Figure 11A

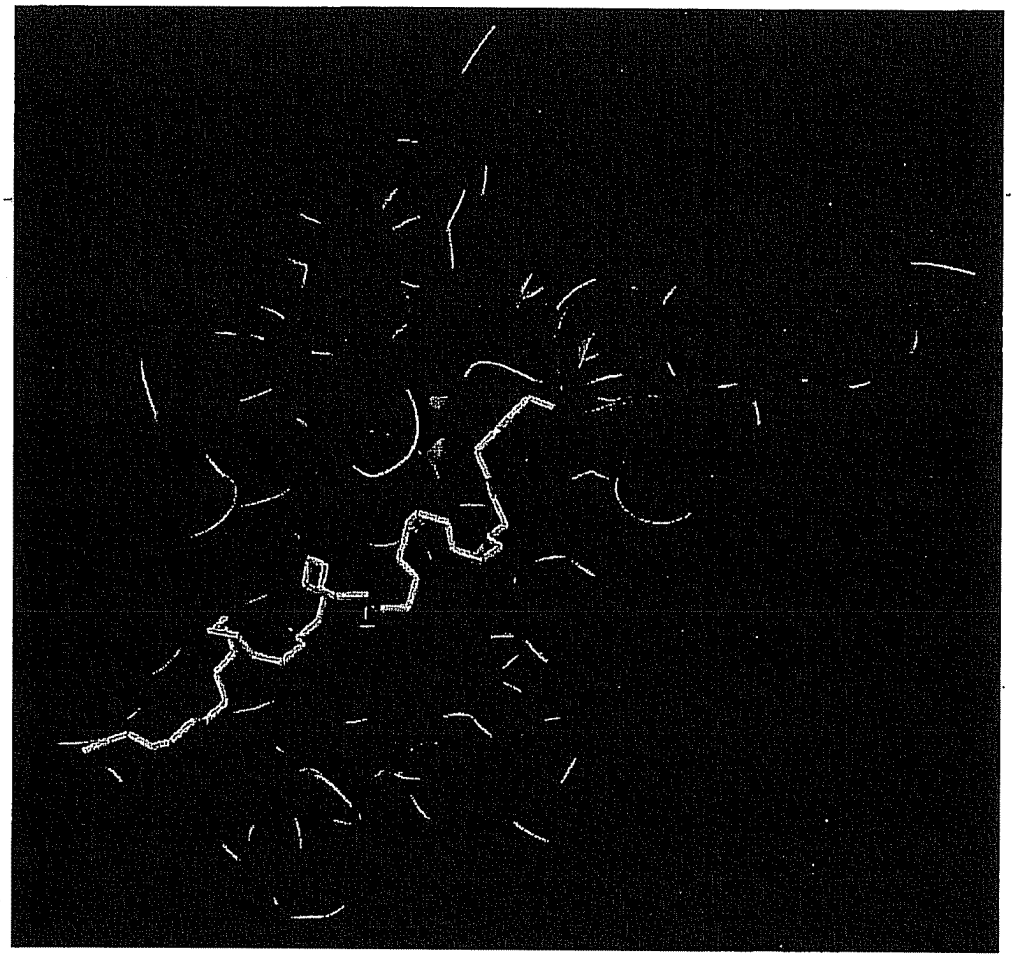


Figure 11B

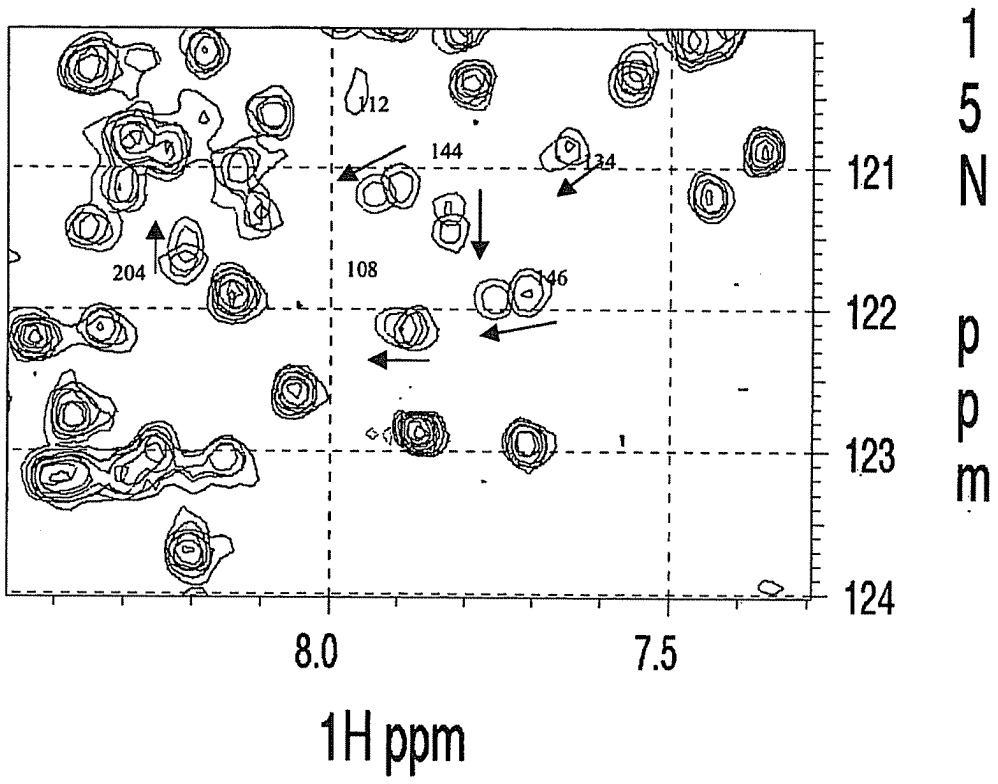


Figure 11C

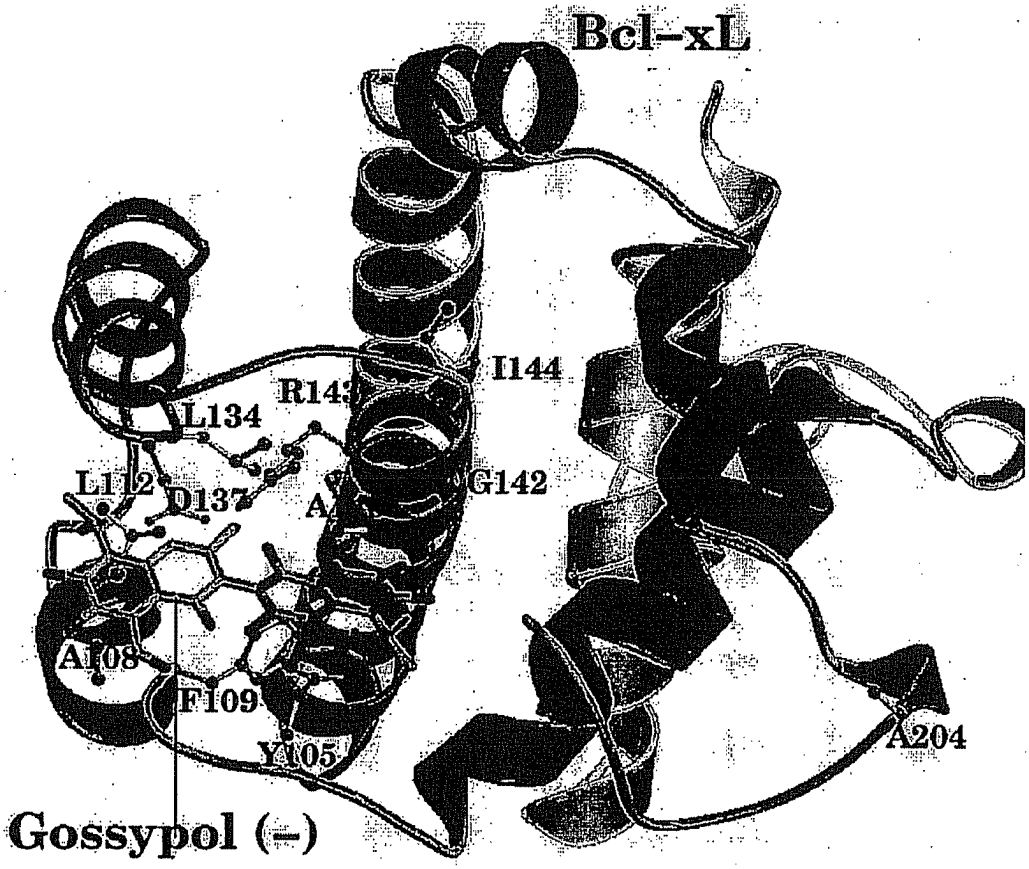


Figure 12

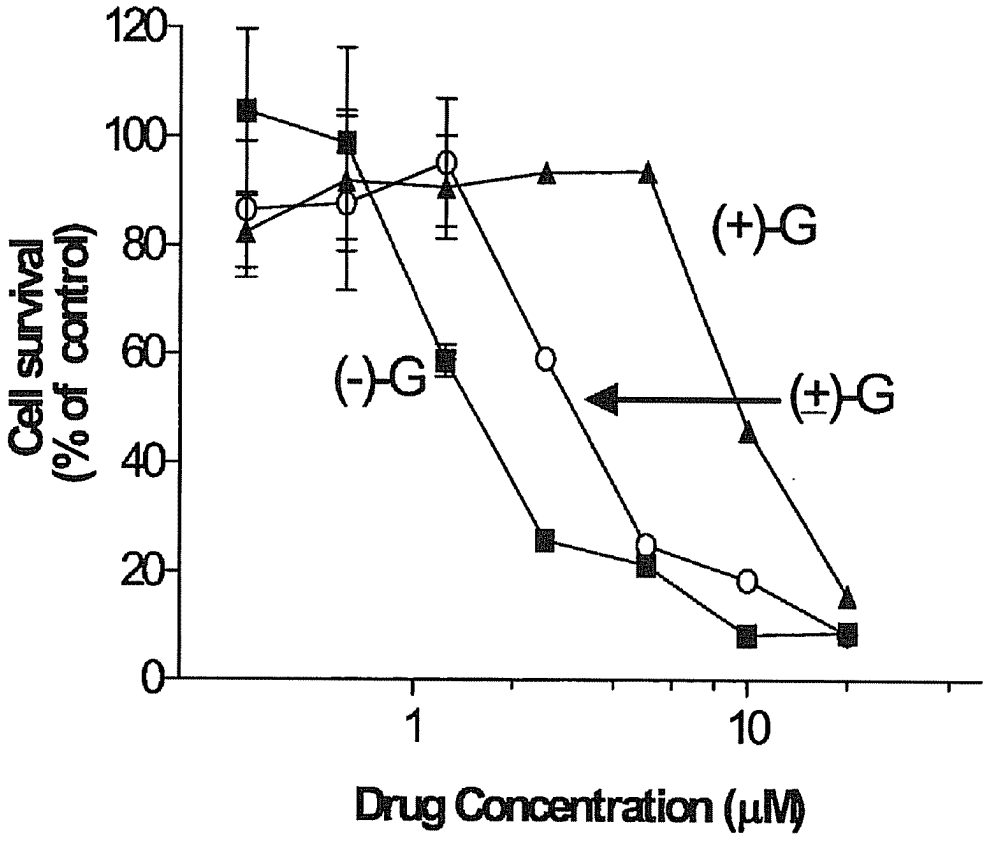

120
100
80
60
40
20
0
Cell survival
(% of control)
(+)-G
(-)-G
(±)-G
1
10
Drug Concentration (μM)

Figure 13

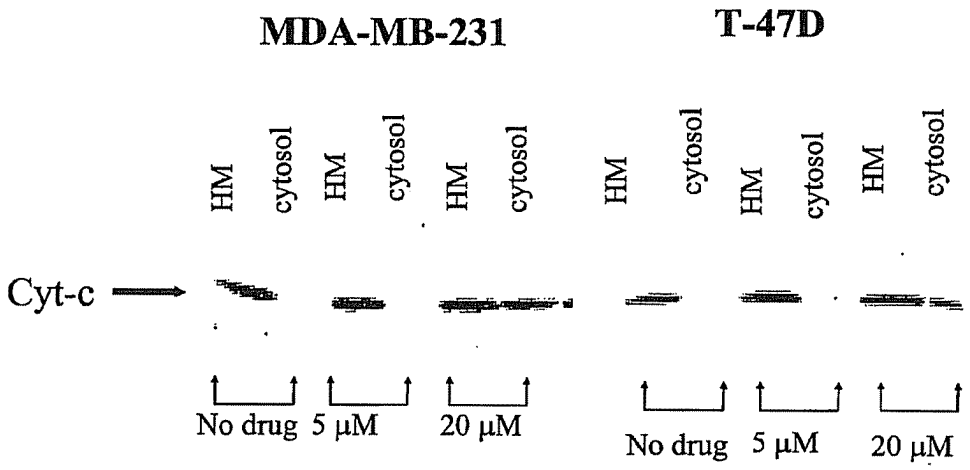

MDA-MB-231
T-47D
HM
cytosol
HM
cytosol
HM
cytosol
HM
cytosol
HM
cytosol
HM
cytosol
Cyt-c
No drug
5 μM
20 μM
No drug
5 μM
20 μM

Figure 14

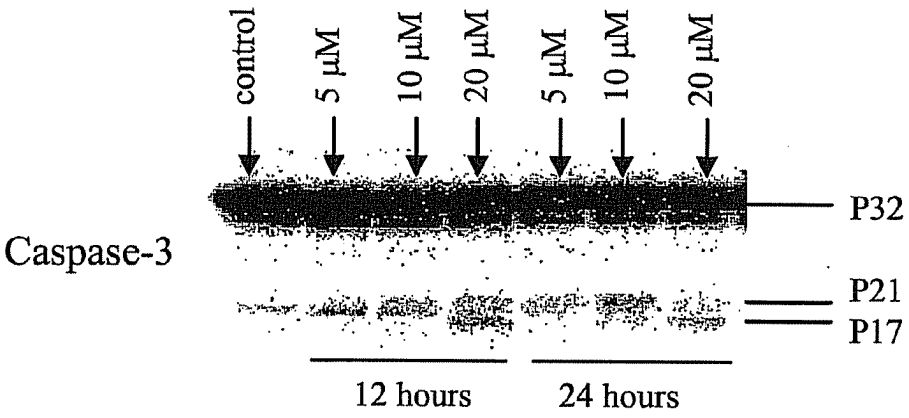

control
5 μM
10 μM
20 μM
5 μM
10 μM
20 μM
Caspase-3
P32
P21
P17
12 hours
24 hours

Figure 15

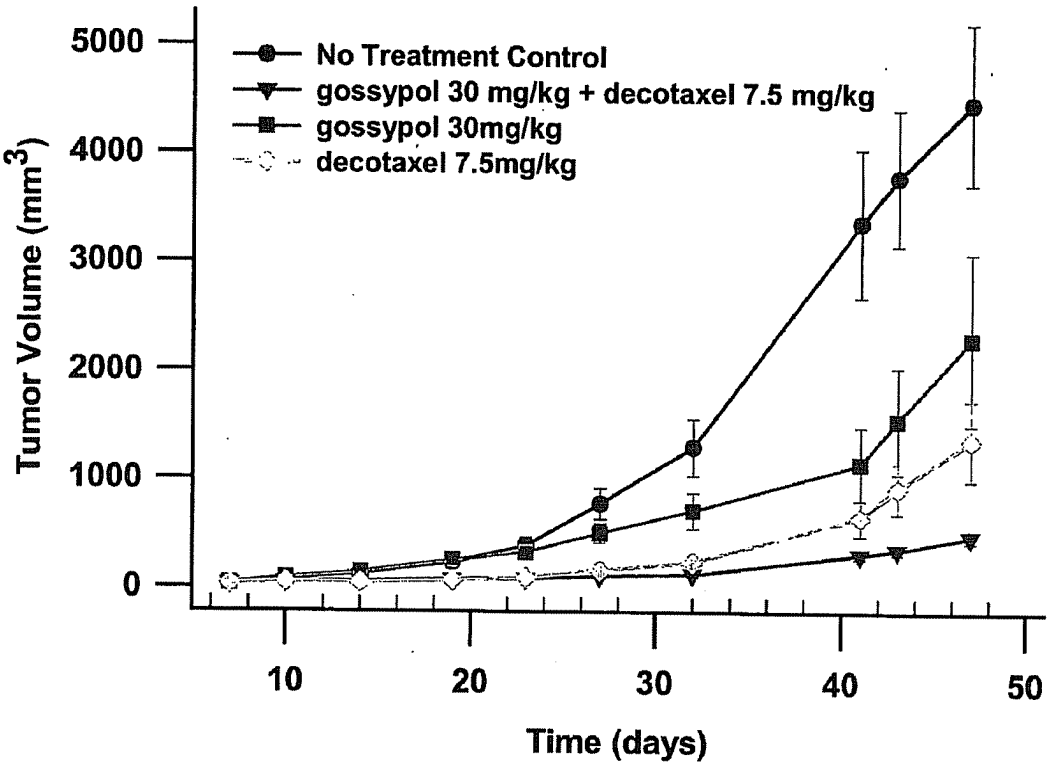
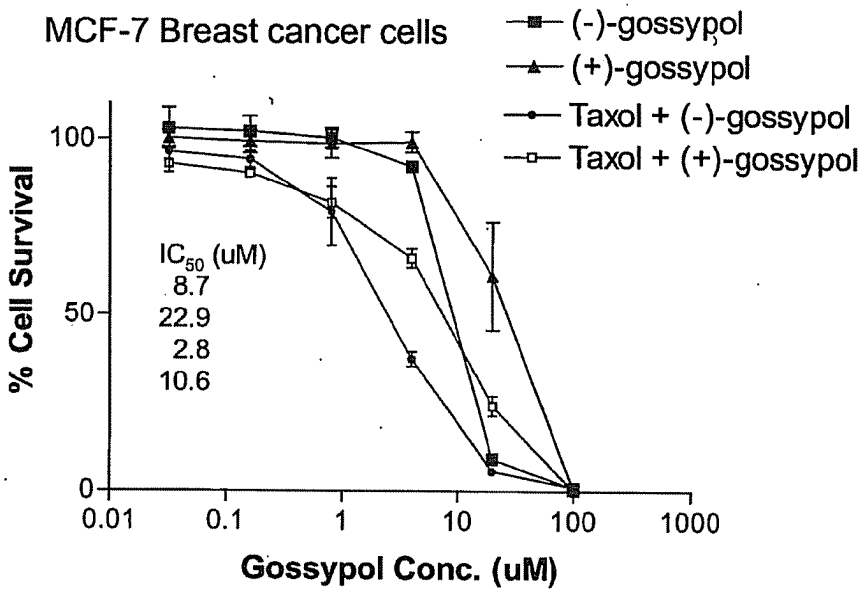

5000
4000
3000
2000
1000
0
Tumor Volume (mm3)
10
20
30
40
50
Time (days)
No Treatment Control
gossypol 30 mg/kg + decotaxel 7.5 mg/kg
gossypol 30mg/kg
decotaxel 7.5mg/kg

Figure 16



This experiment used 100:1 ratio between (-)-gossypol and Taxol, and between (+)-gossypol and Taxol

Figure 17A

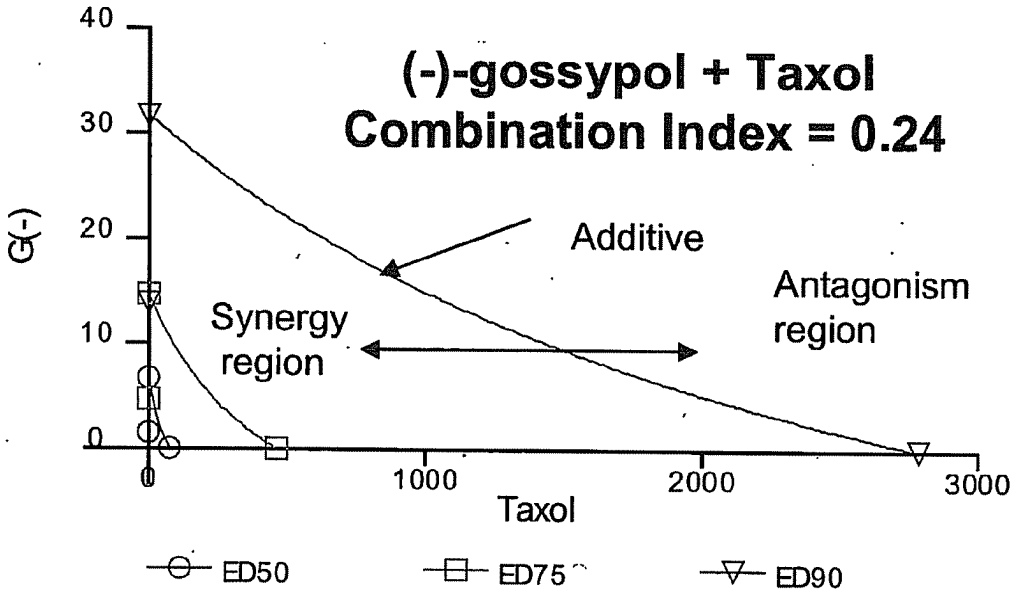

(-)-gossypol + Taxol
Combination Index = 0.24
G(-)
40
30
20
10
0
0
1000
2000
3000
Taxol
Additive
Synergy region
Antagonism region
ED50
ED75
ED90

Figure 17B

MDA-MB-231 DOX + G- 1:2.5uM

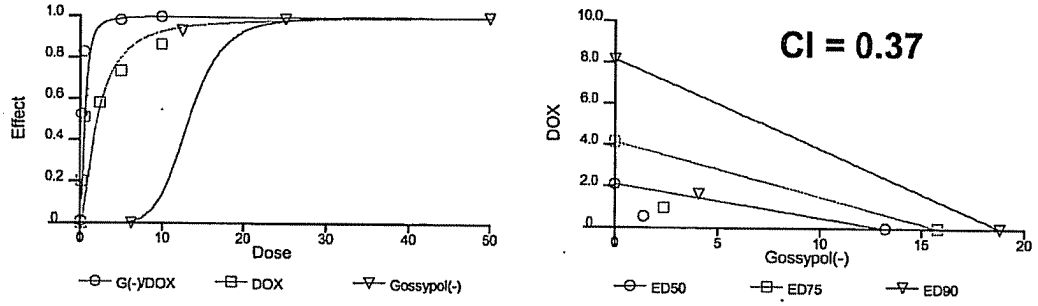


Figure 18

Effect of (-)-gossypol on inhibition of tumor growth of human breast cancer xenograft MDA-231

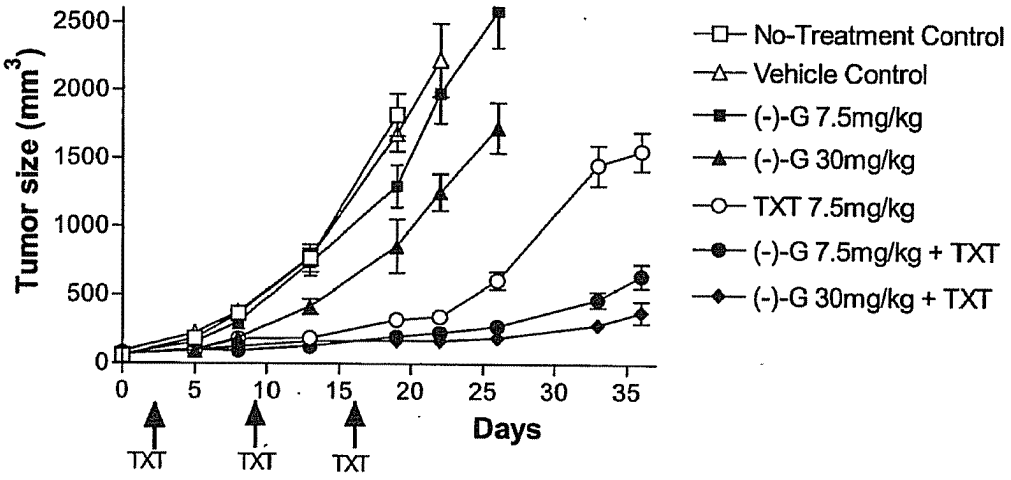


Figure 19

Effect of (-)-gossypol on inhibition of tumor growth of human breast cancer xenograft MDA-231

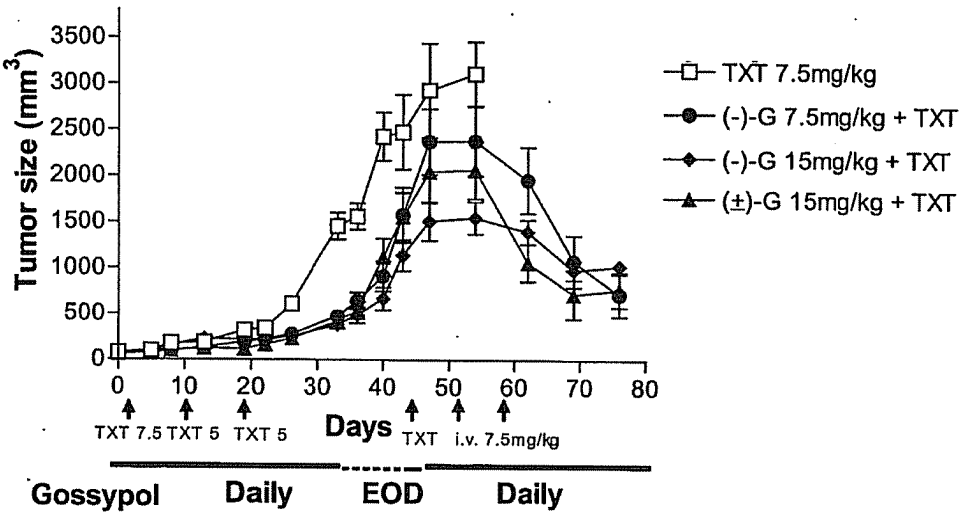


Figure 20

Effect of (-)-gossypol on inhibition of tumor growth of human non-samll cell lung carcinoma cell xenograft A-549

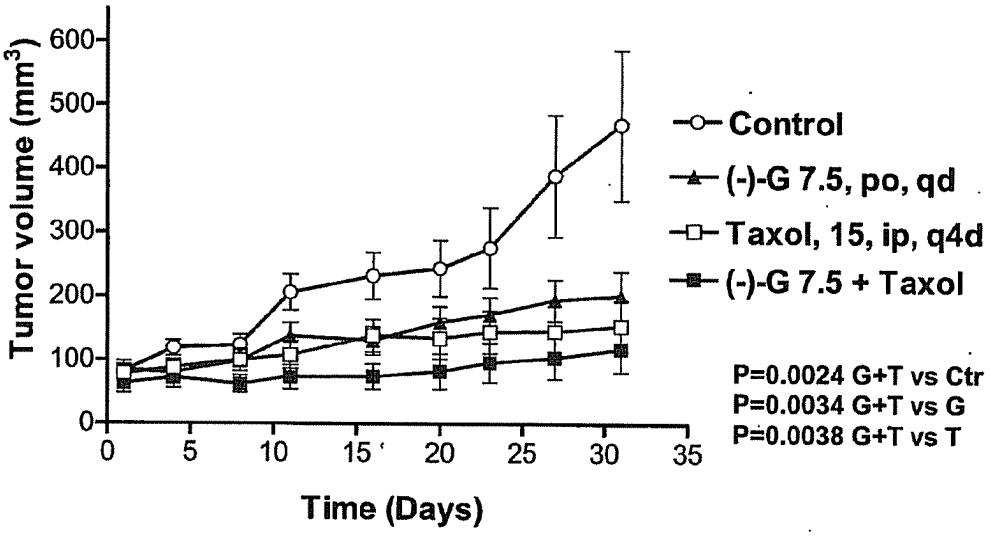


Figure 21

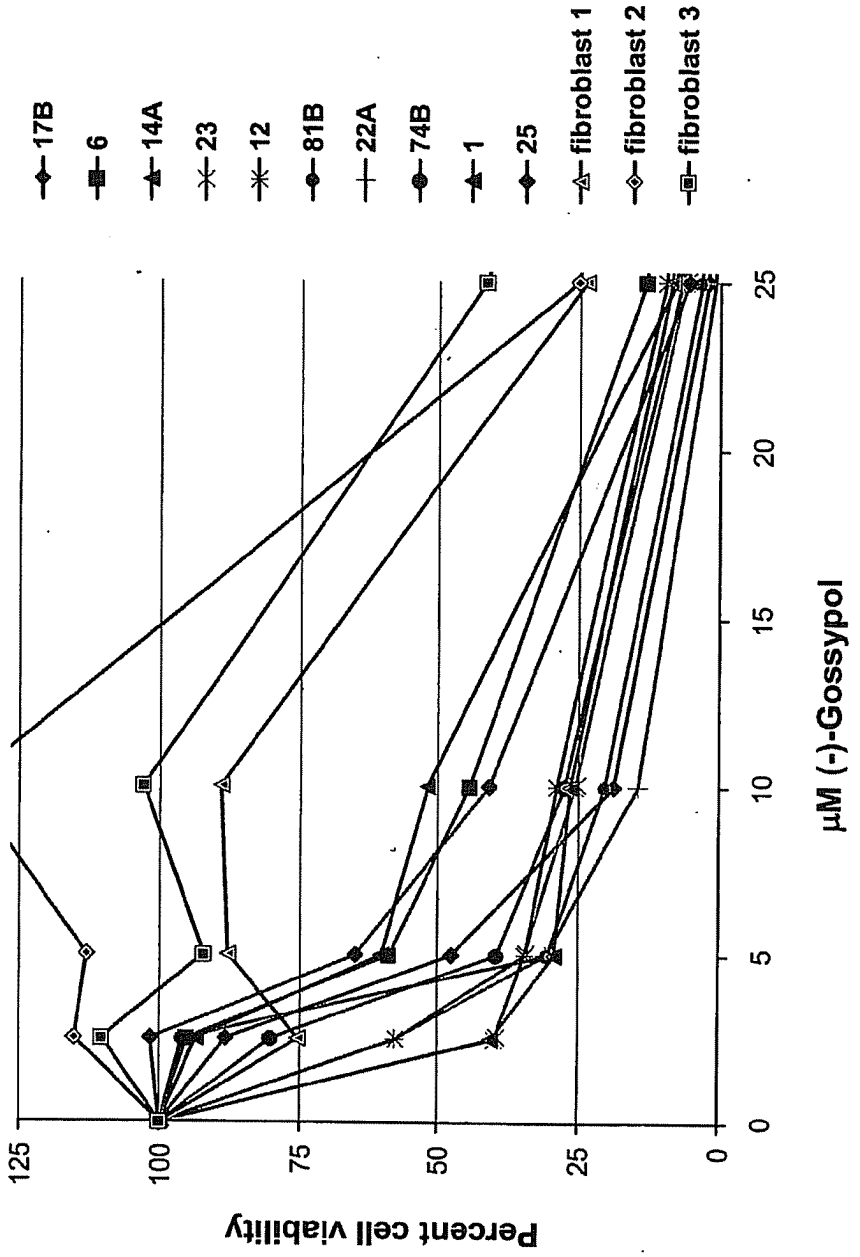

17B
6
14A
23
12
81B
22A
74B
1
25
fibroblast 1
fibroblast 2
fibroblast 3
Percent cell viability
125
100
75
50
25
0
0
5
10
15
20
25
µM (-)-Gossypol

Figure 22

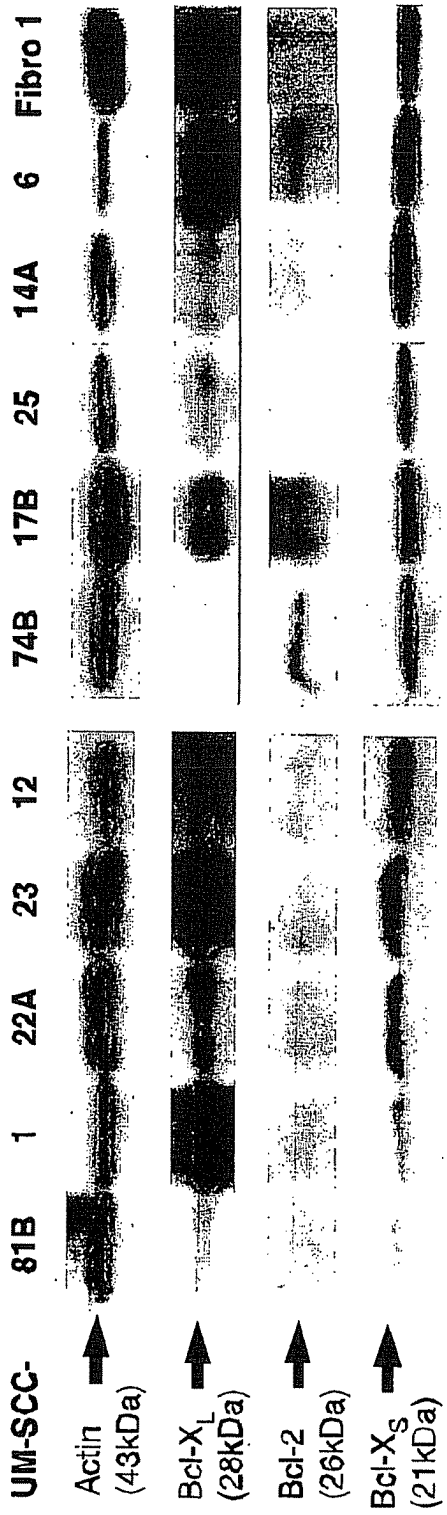

UM-SCC-
81B
1
22A
23
12
74B
17B
25
14A
6
Fibro 1
Actin (43kDa)
Bcl-X$_L$ (28kDa)
Bcl-2 (26kDa)
Bcl-X$_S$ (21kDa)

Figure 23

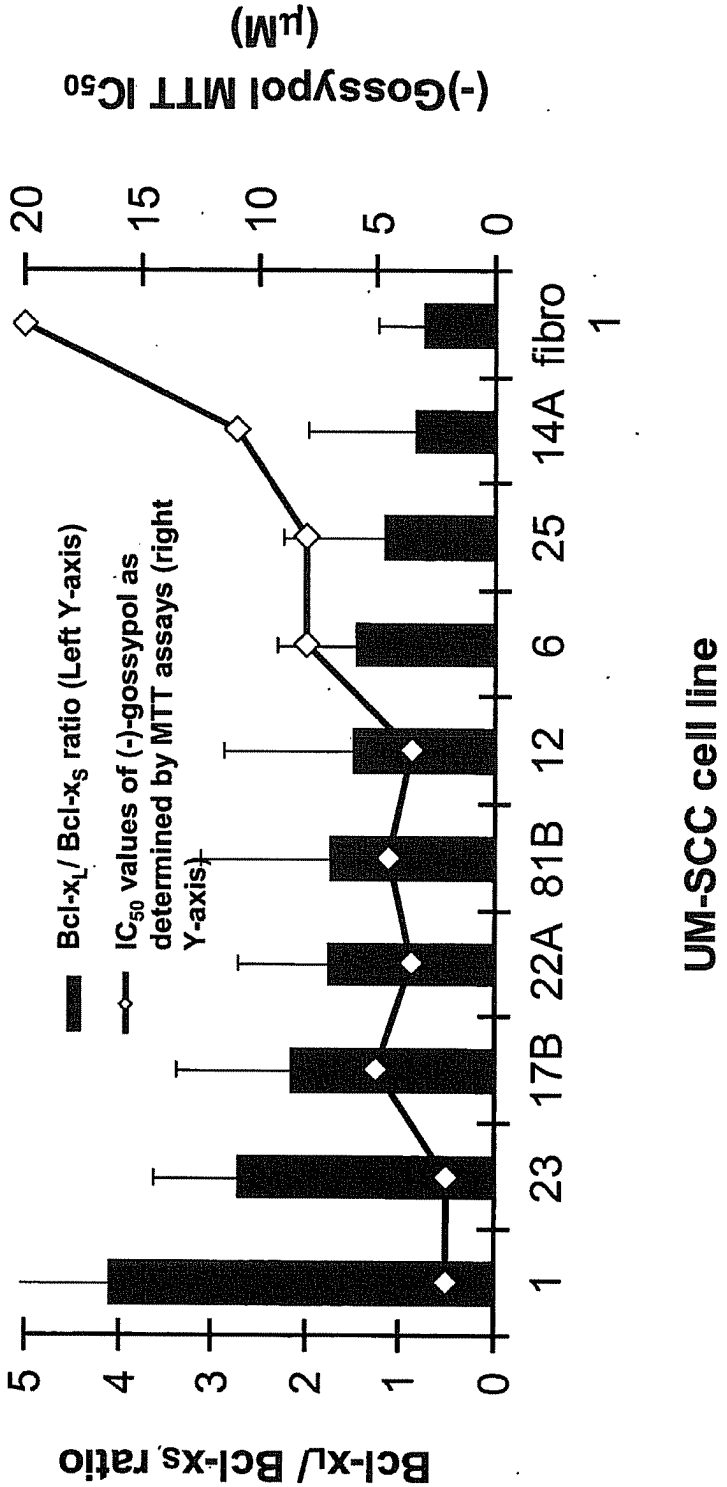

Bcl-xL/ Bcl-xs ratio
5
4
3
2
1
0
1
23
17B
22A
81B
12
6
25
14A
fibro
1
UM-SCC cell line
20
15
10
5
0
(-)Gossypol MTT IC50 (μM)
Bcl-xL/ Bcl-xS ratio (Left Y-axis)
IC50 values of (-)-gossypol as determined by MTT assays (right Y-axis)

Figure 24A

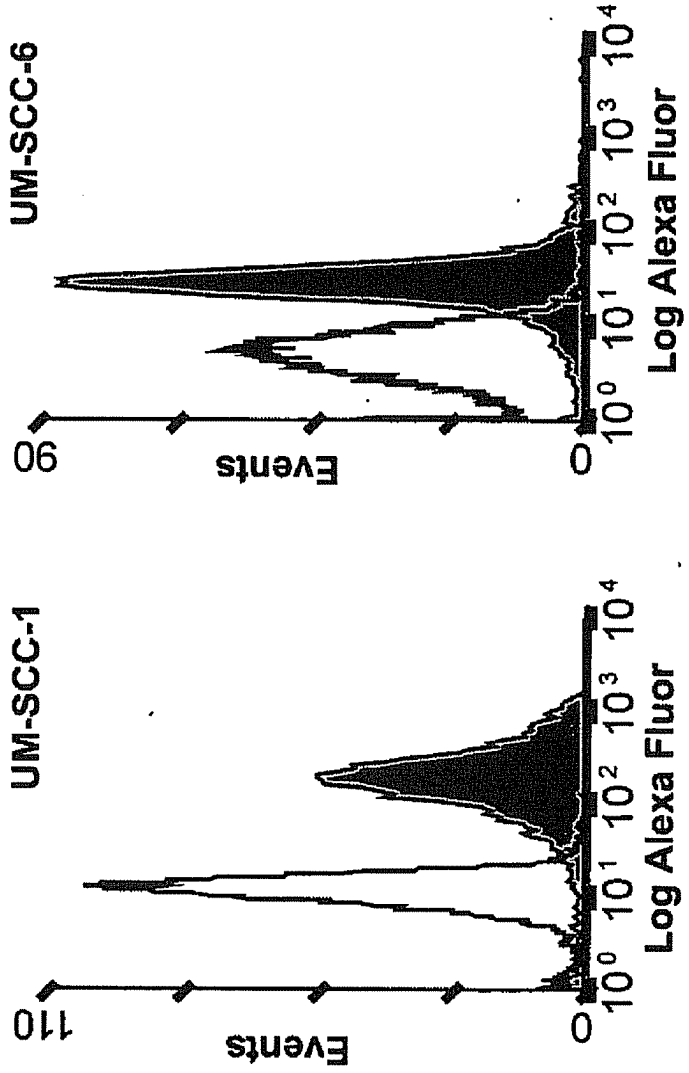

UM-SCC-6
Events
90
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor
UM-SCC-1
Events
110
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor

Figure 24B

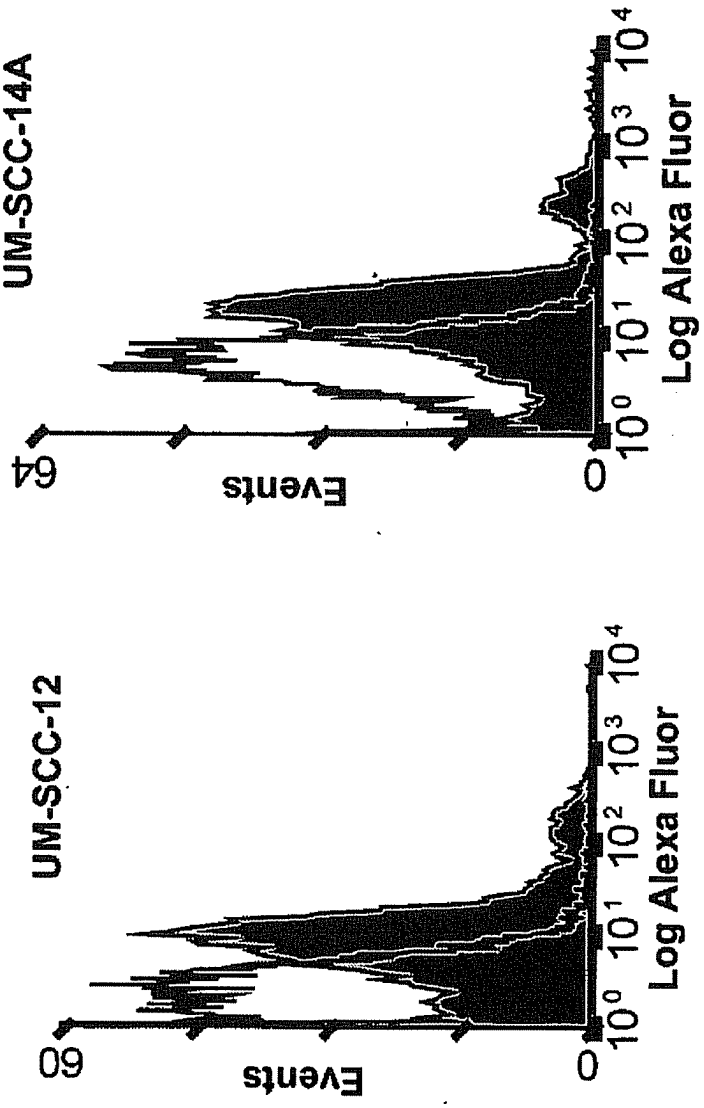

UM-SCC-14A
64
Events
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor
UM-SCC-12
60
Events
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor

Figure 24C

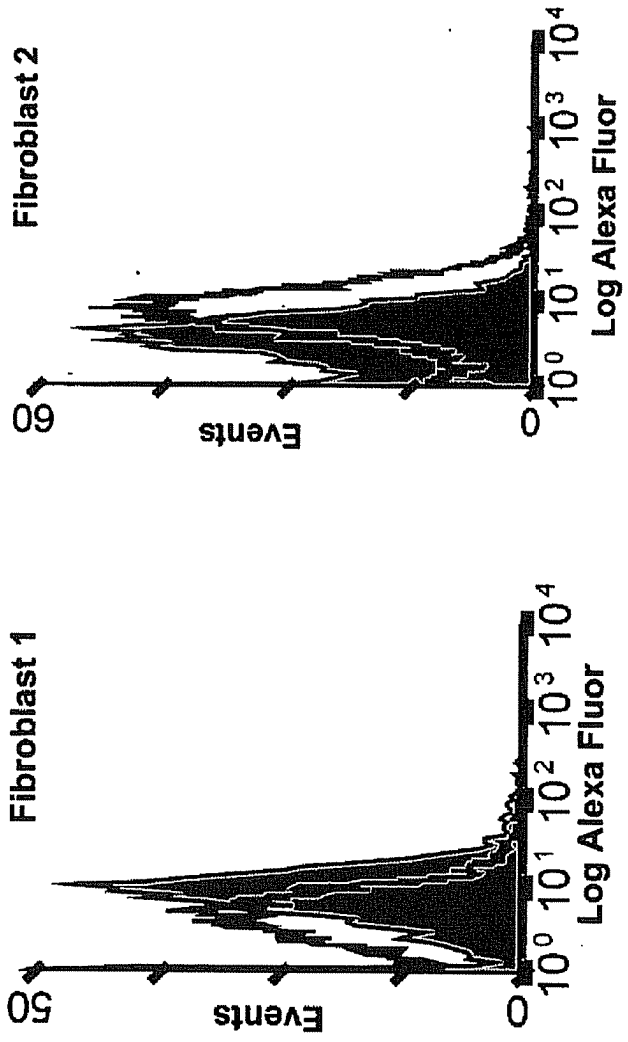
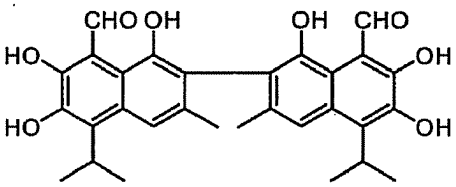
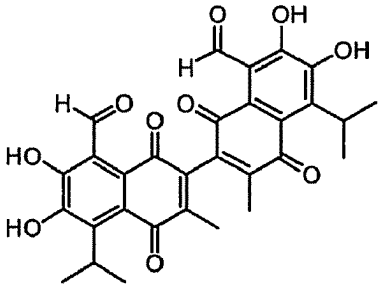

Fibroblast 2
Events
60
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor
Fibroblast 1
Events
50
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor

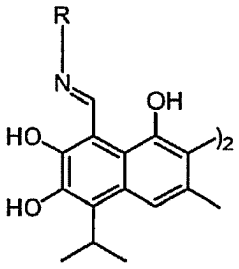
Figure 25



Gossypol

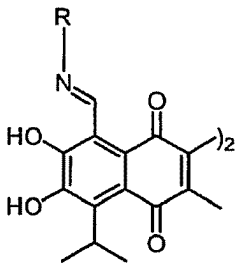


Gossypolone



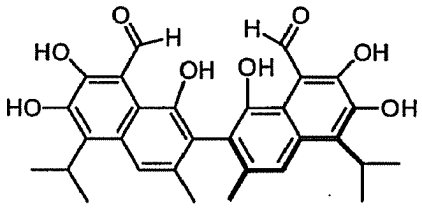
R = aliphatic or aromatic group

Schiff's base of Gossypol

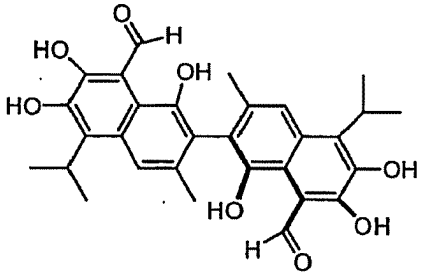


R = aliphatic or aromatic group

Schiff's base of Gossypolone



(-)-(*R*)-Gossypol



(+)-(*S*)-Gossypol

Figure 26

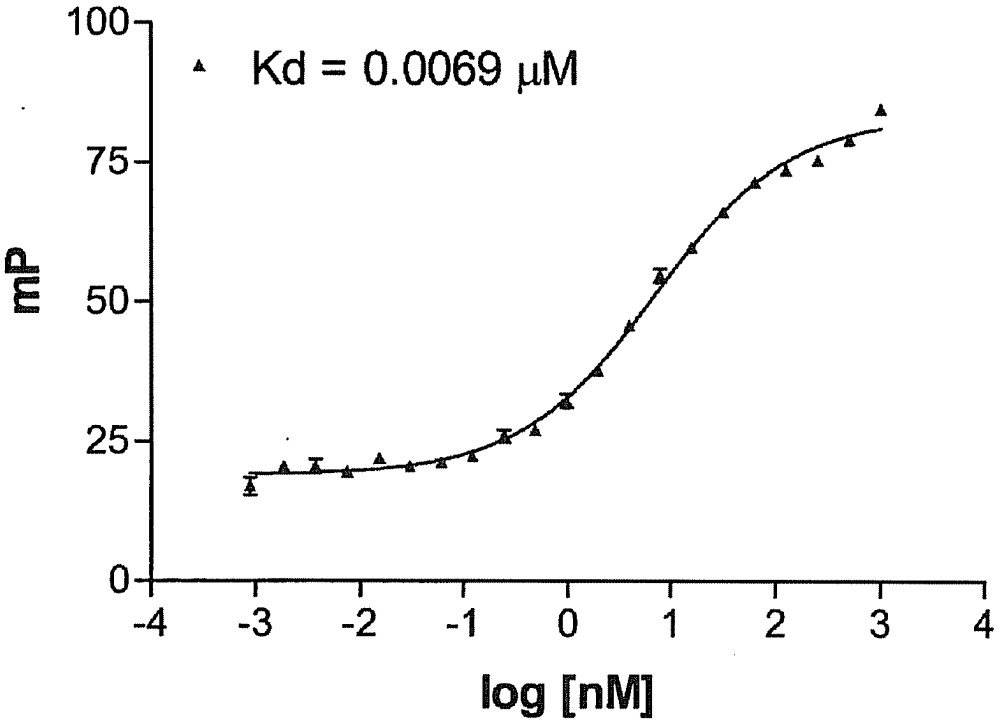


Figure 27

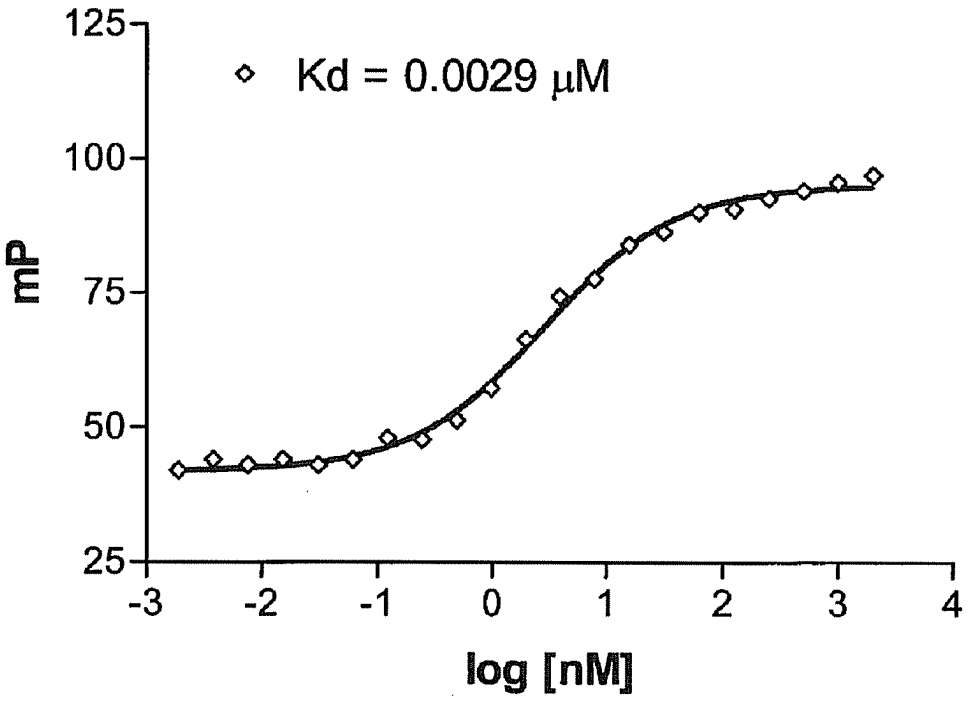


Figure 28A

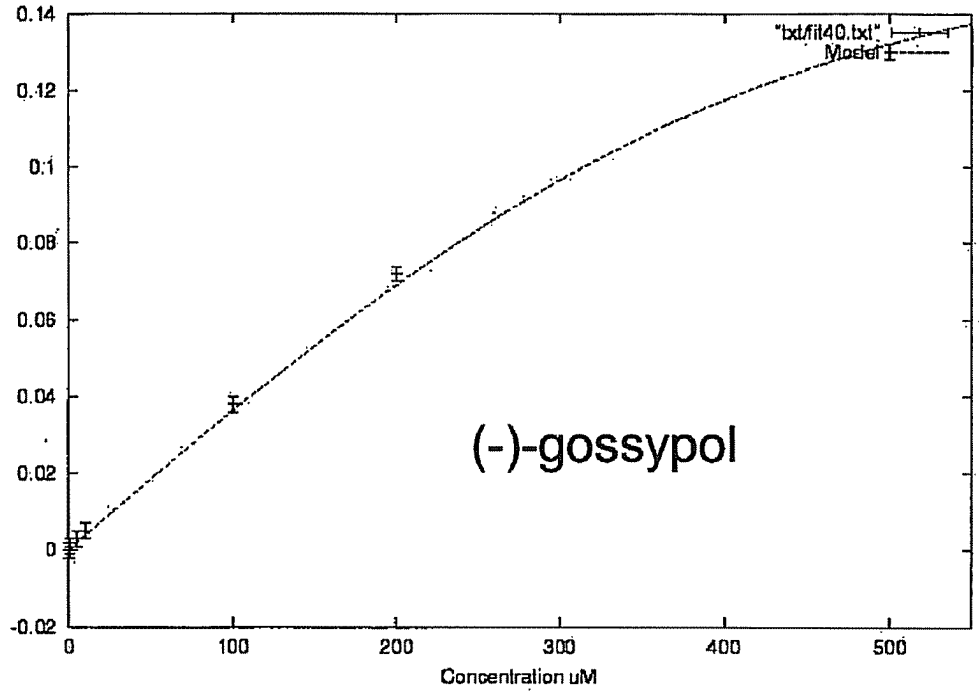

0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
0
100
200
300
400
500
Concentration uM
"txt/fit40.txt"
Model
(-)-gossypol

Figure 28B

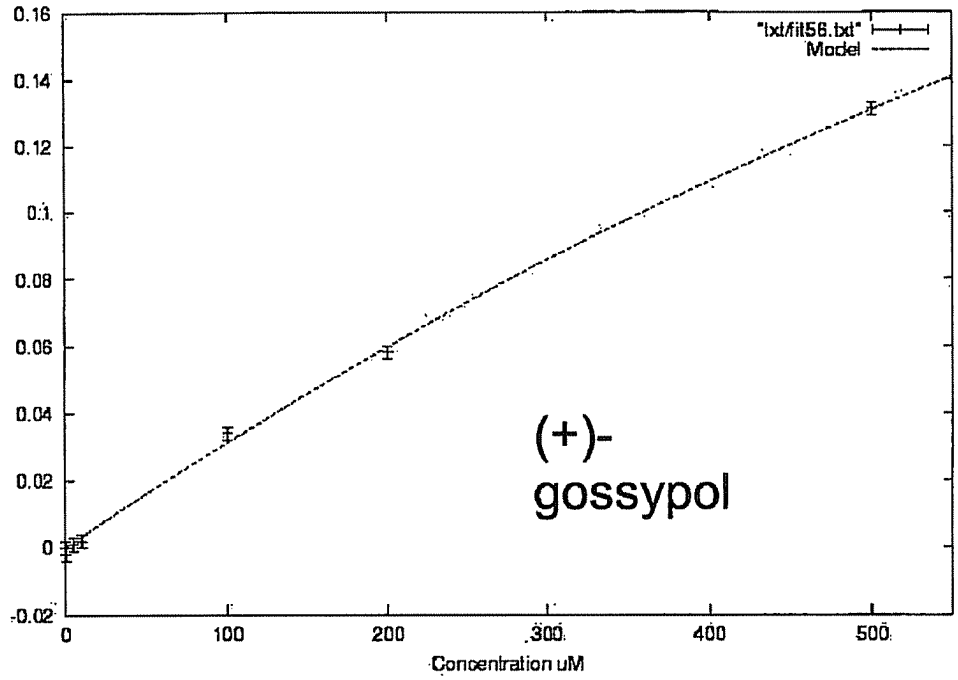

"txt/fit56.txt"
Model
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
0
100
200
300
400
500
Concentration uM
(+)-
gossypol

Figure 29

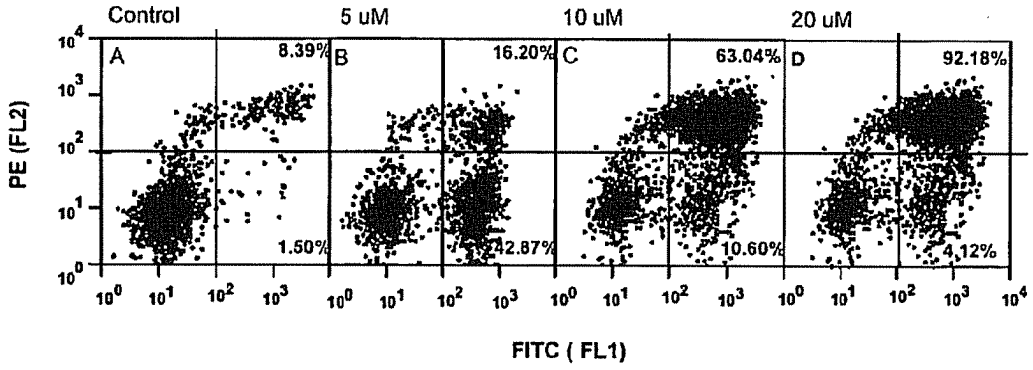

Control
5 uM
10 uM
20 uM
A
8.39%
1.50%
B
16.20%
2.87%
C
63.04%
10.60%
D
92.18%
4.12%
PE (FL2)
FITC (FL1)

Figure 30

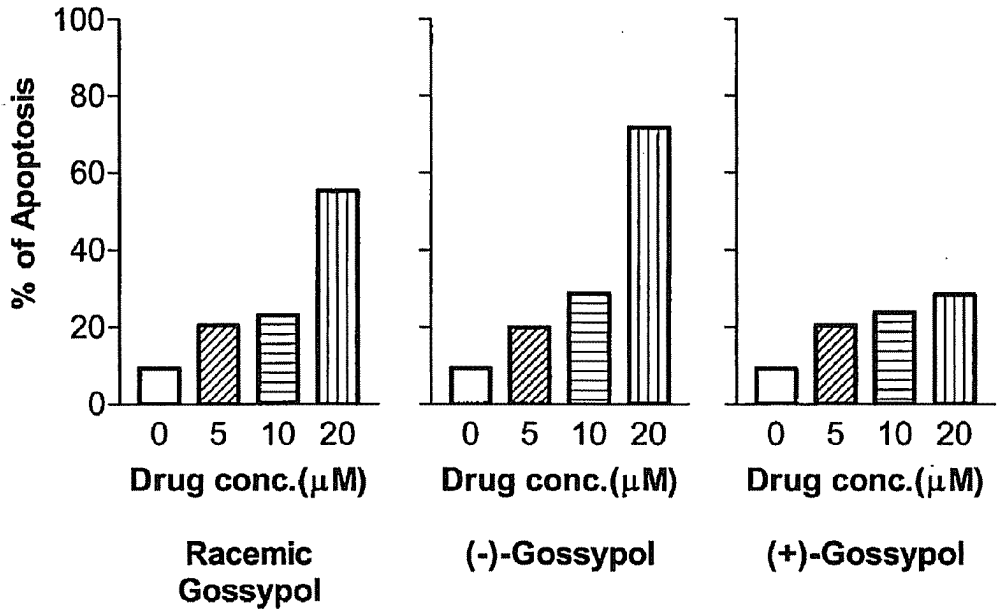

% of Apoptosis
100
80
60
40
20
0
0 5 10 20
Drug conc.(μM)
Racemic Gossypol
0 5 10 20
Drug conc.(μM)
(-)-Gossypol
0 5 10 20
Drug conc.(μM)
(+)-Gossypol

Figure 31

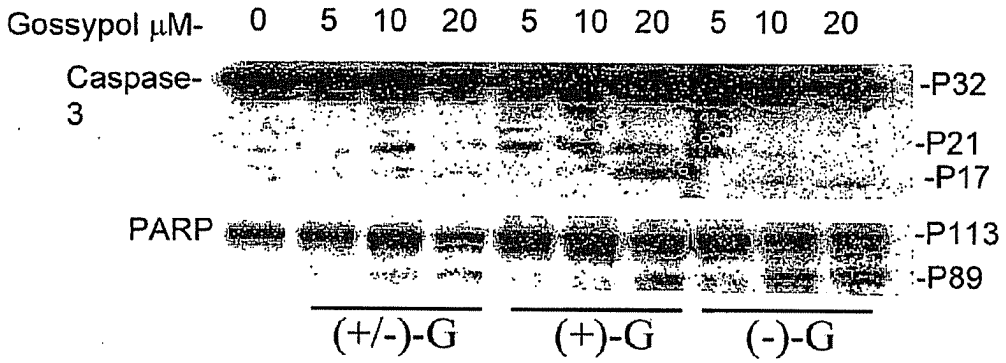

Gossypol μM- 0 5 10 20 5 10 20 5 10 20
Caspase-3
-P32
-P21
-P17
PARP
-P113
-P89
(+/-)-G
(+)-G
(-)-G

Figure 32A

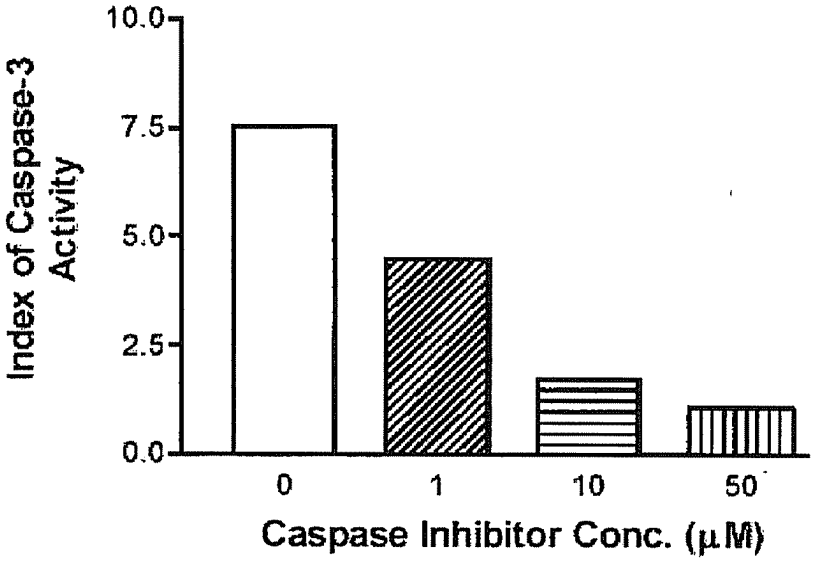


Figure 32B

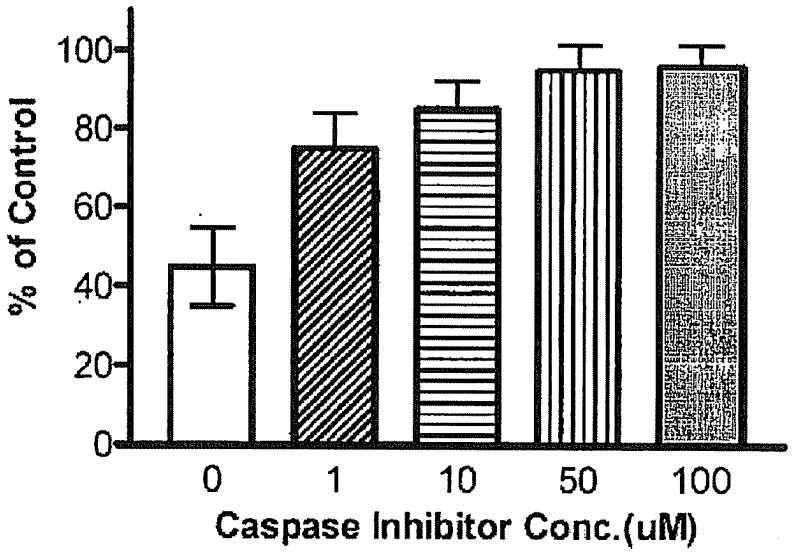


Figure 33

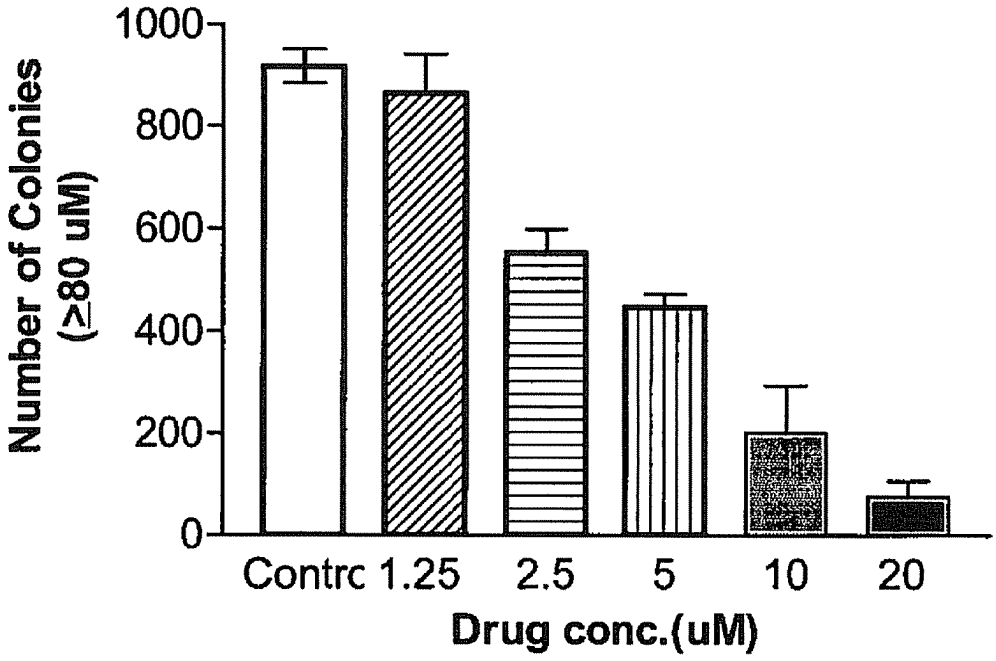

Number of Colonies (≥80 uM)
1000
800
600
400
200
0
Contrc
1.25
2.5
5
10
20
Drug conc.(uM)

Figure 34

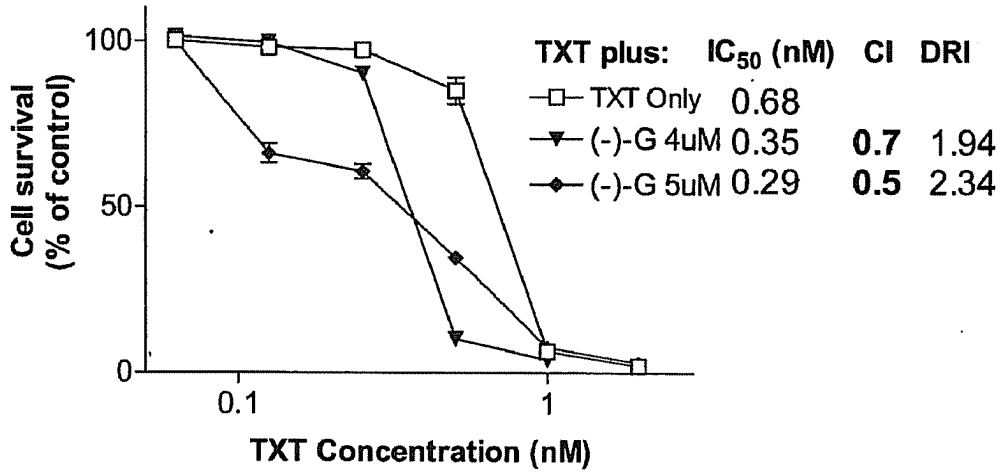

Cell survival (% of control)
100
50
0
0.1
1
TXT Concentration (nM)
TXT plus: IC50 (nM) CI DRI
TXT Only 0.68
(-)-G 4uM 0.35 0.7 1.94
(-)-G 5uM 0.29 0.5 2.34

Figure 35A

In vitro effects of gossypol-(-) in combination with various doses of radiation on PC-3 clonogenic assays

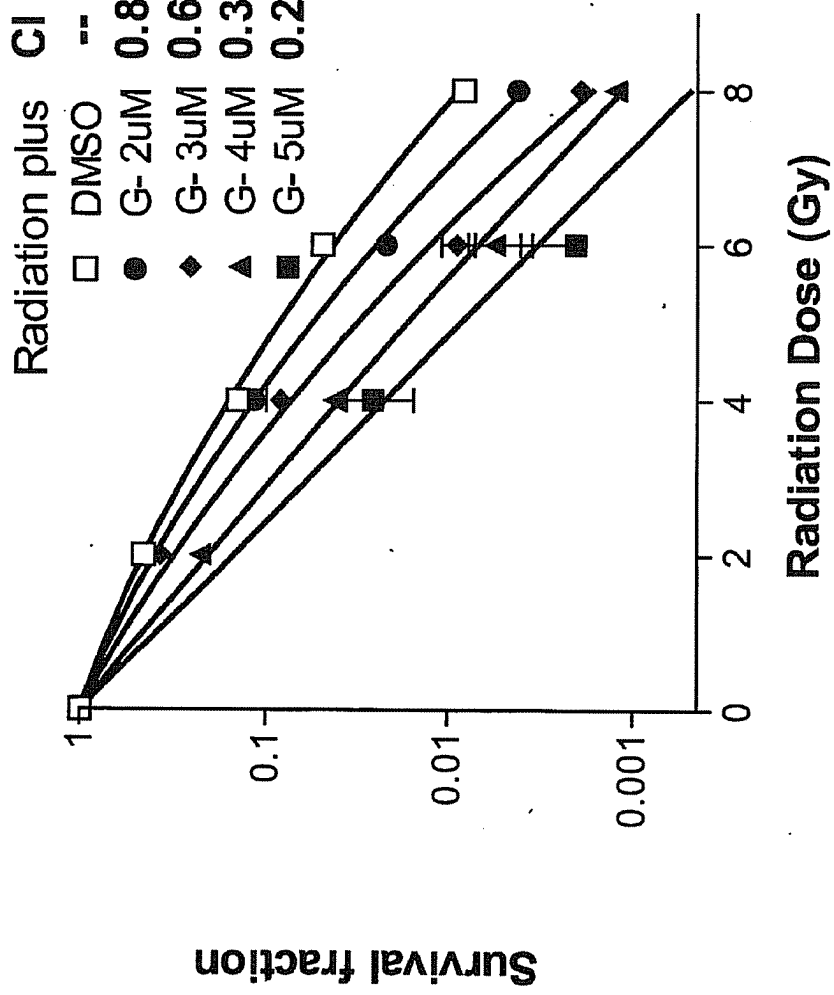


Figure 35B

G- μM	0	1	2	3	4	5
D bar = Mean inactivation dose	2.22	2.06	1.95	1.63	1.26	1.05
Gy(1%)= Dose required for 1% cell survival	7.84	7.11	7.03	6.25	5.59	4.84
SF(2Gy)= Survival fraction at 2Gy	0.45	0.43	0.4	0.31	0.21	0.15

Figure 36

(-)-gossypol in combination with radiation in an androgen-independent prostate PC-3 xenograft model

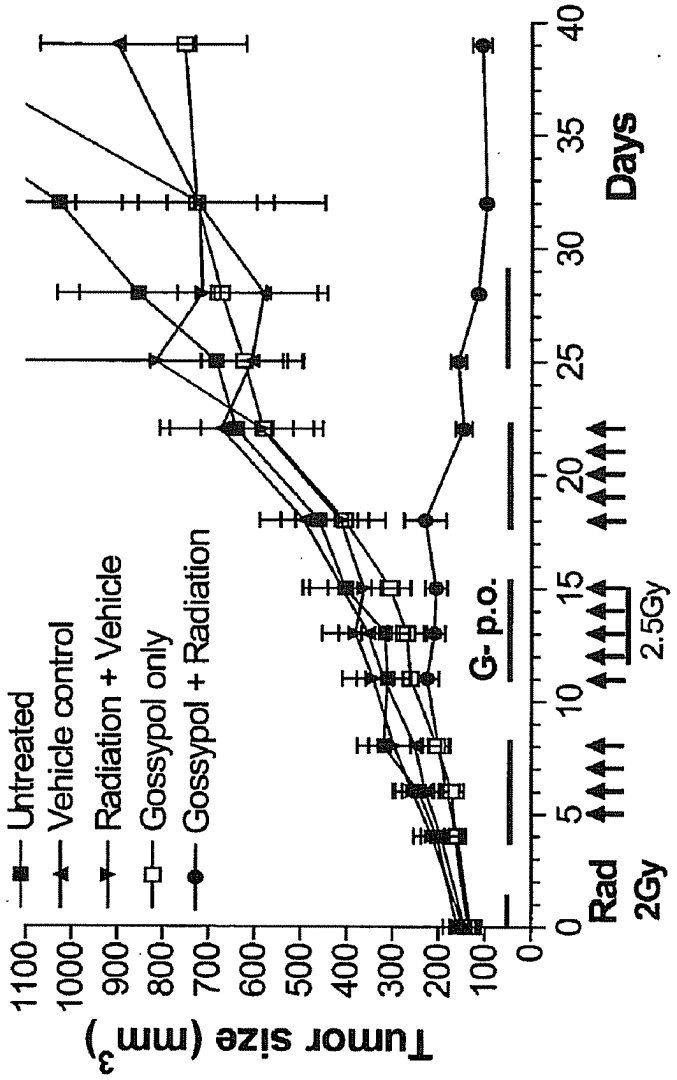


Figure 37

(-)-gossypol in combination with radiation in an androgen-independent prostate PC-3 xenograft model

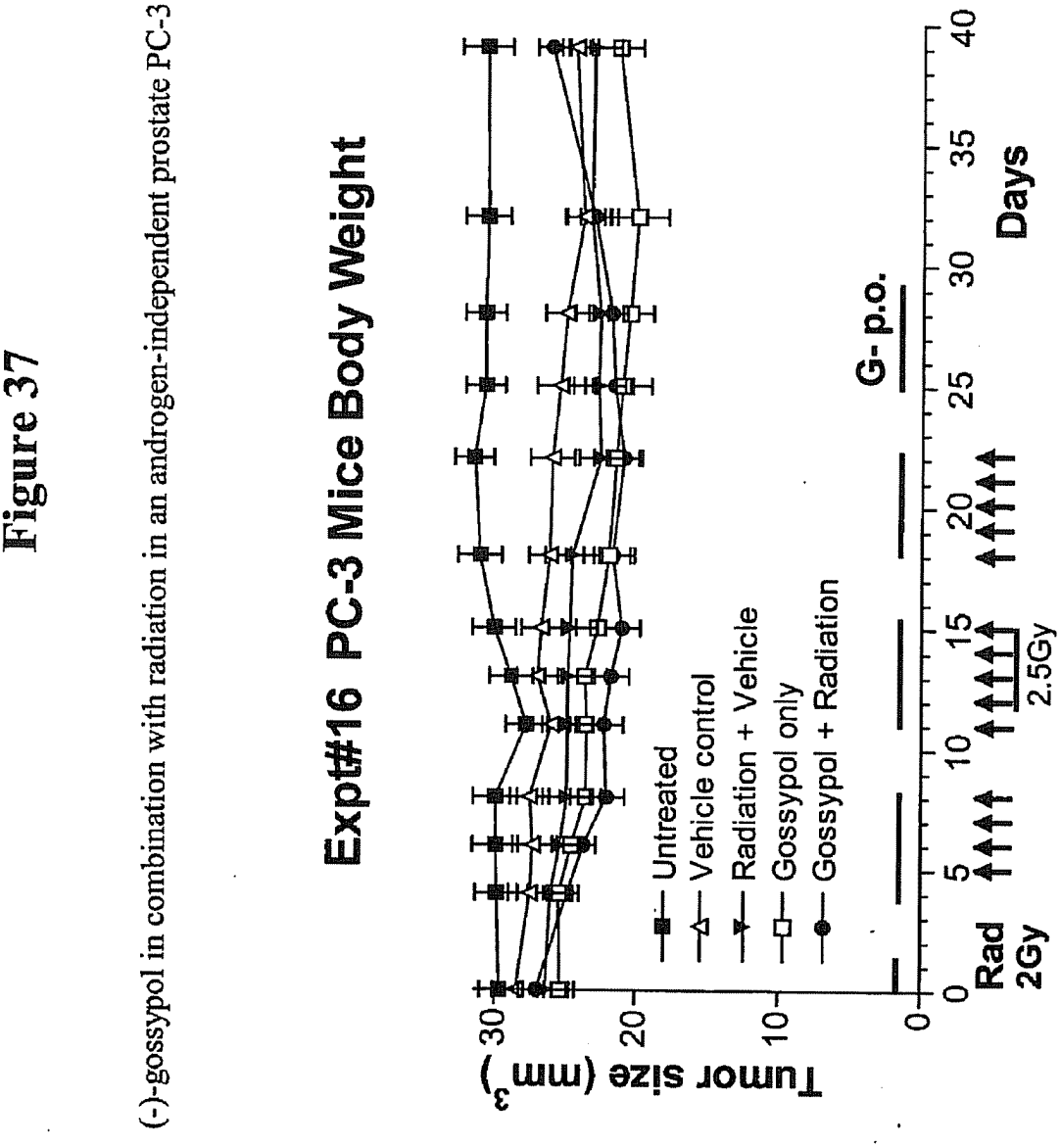


Figure 38

(-)-gossypol in combination with radiation in an androgen-independent prostate PC-3 xenograft model

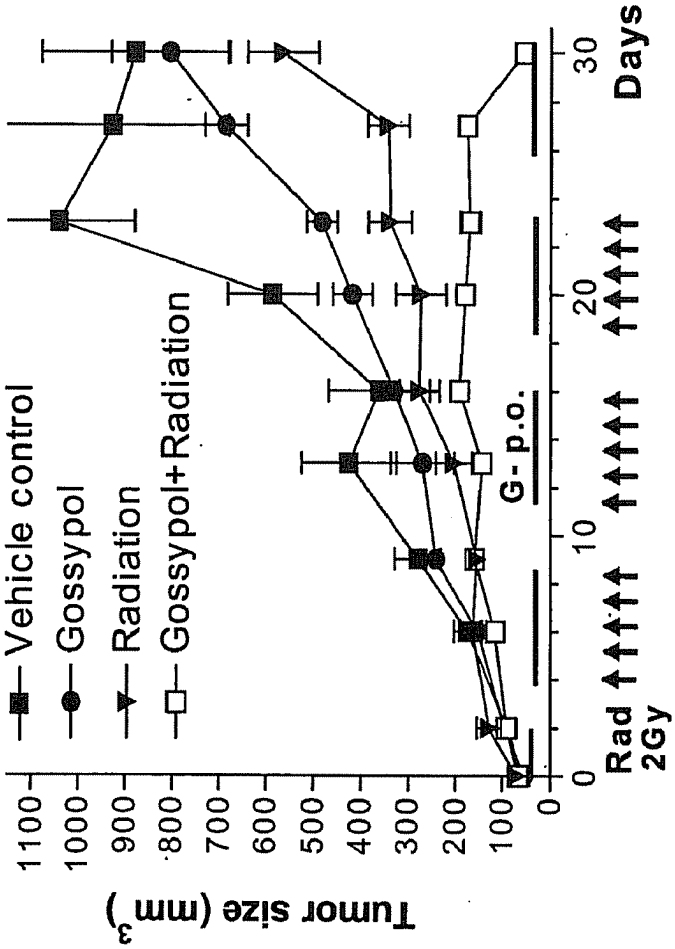
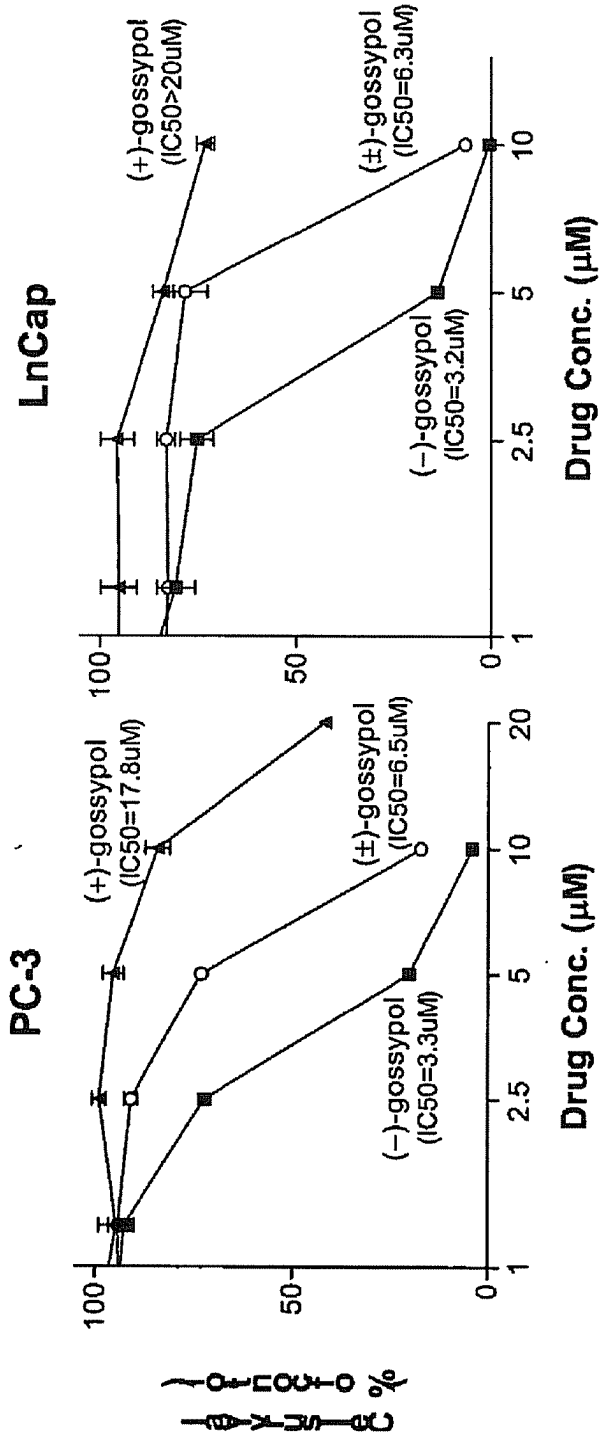
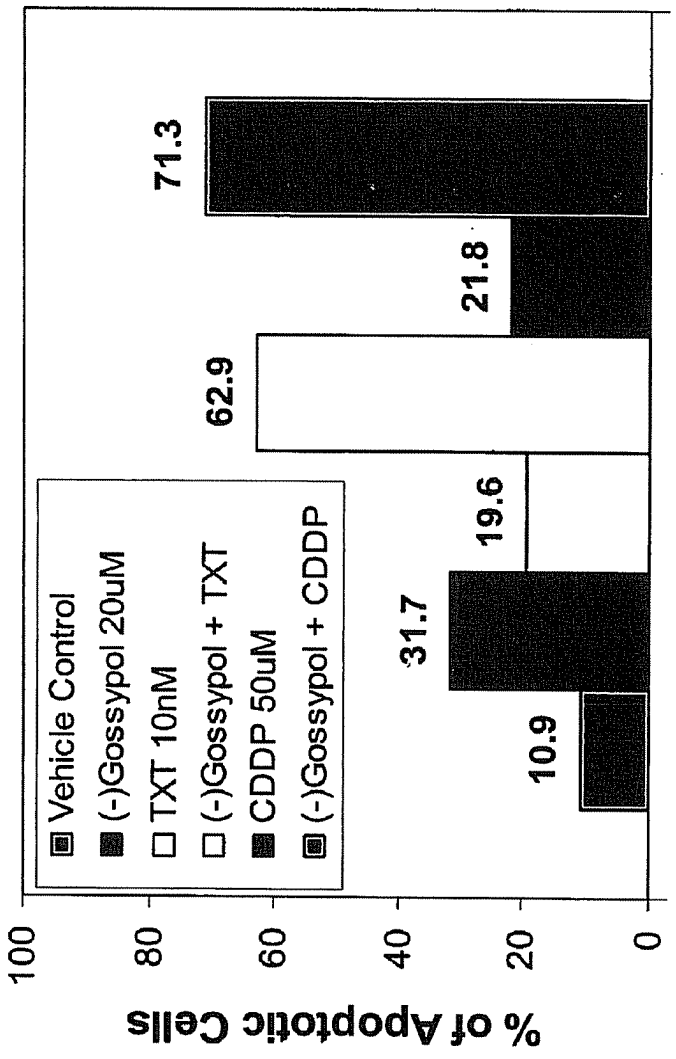


Figure 39



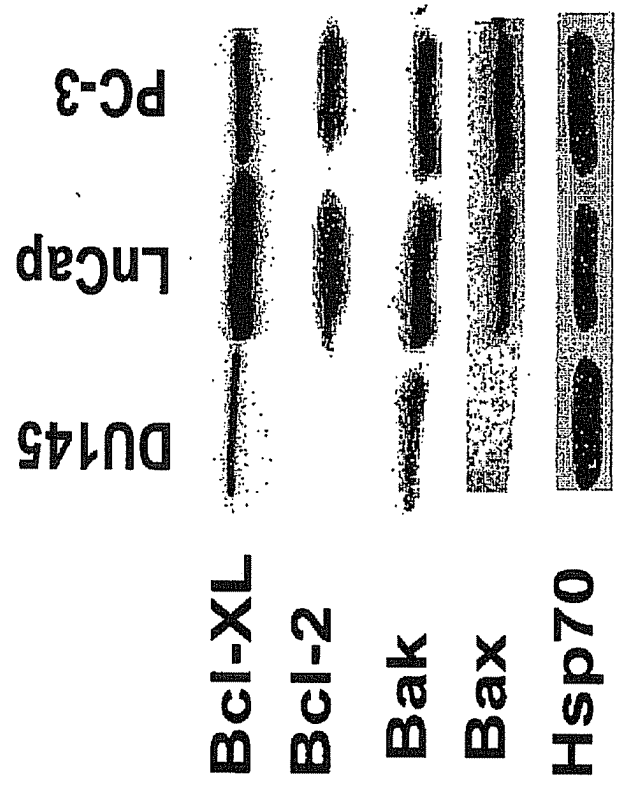
Prostate cancer cell growth inhibition by gossypol. PC-3 and LnCap cells in 96-well plates were treated in triplicates with gossypol and its enantiomers. MTT-based 5-day cell proliferation assay was performed and IC50, drug concentration that inhibited 50% of cell growth, was calculated. (-)-gossypol is 5-10 times more potent that (±)-gossypol, 2 times more potent than (±)-gossypol, in both cell lines.

Figure 40



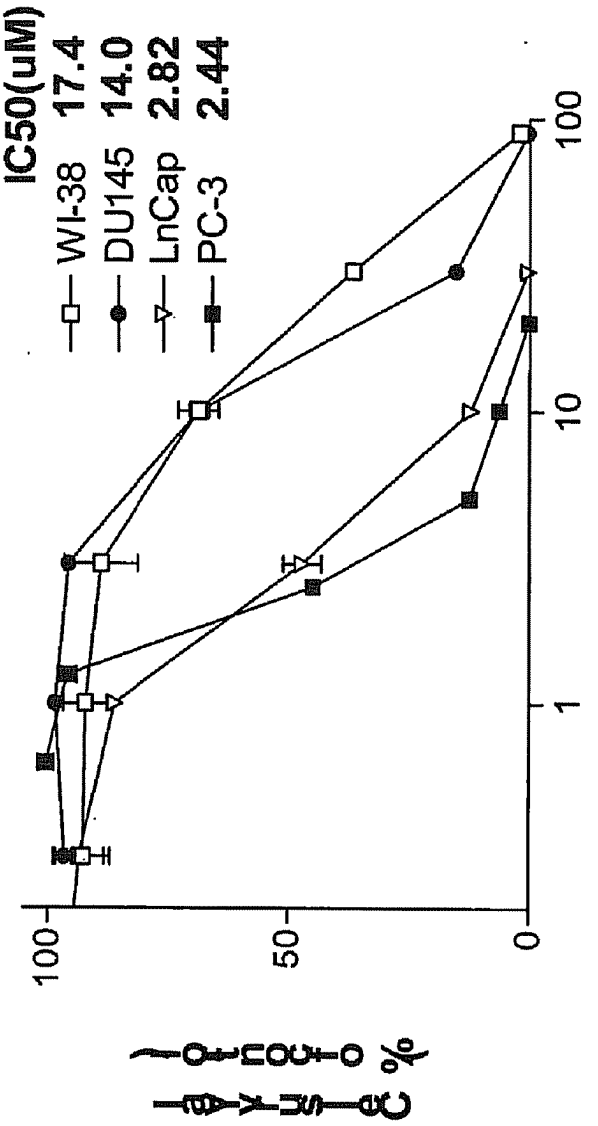
(-)-Gossypol enhances chemotherapy-induced apoptosis in human prostate cancer PC-3 cells. Cells were treated with (-)-gossypol alone or in combination with TXT or CDDP for 48hr, then stained with Annexin V-FITC and PI for flow cytometry. Values are % of apoptotic cells.

Figure 41

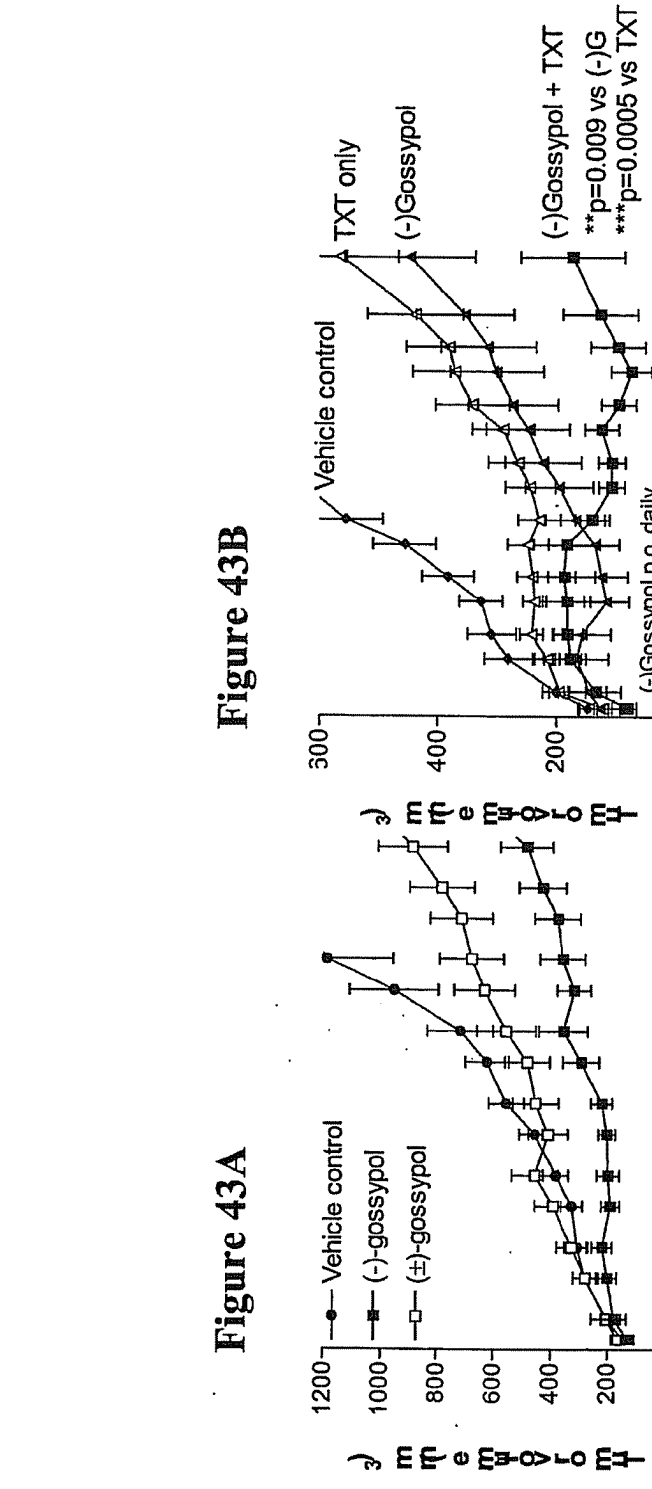


Basal levels of Bcl-2 family proteins expression in three prostate cancer cell lines. HSP70: heat shock protein 70kDa for gel loading control.

Figure 42



Cytotoxicity of (-)-gossypol on prostate cancer cells. MTT-based 5-day cell proliferation assay was performed and IC50, drug concentration that inhibited 50% of cell growth, was calculated.



In vivo anti-tumor activity of gossypol in human prostate cancer PC-3 xenograft model. **A:** 15mg/kg (±)- or (-)-gossypol p.o. daily for 26 days. (-)-gossypol is more potent than (±)-gossypol ($P<0.001$). **B:** Tumor growth inhibition by (-)-gossypol was significantly enhanced when used in combination with docetaxel (TXT). **Student's t-test.

Figure 44

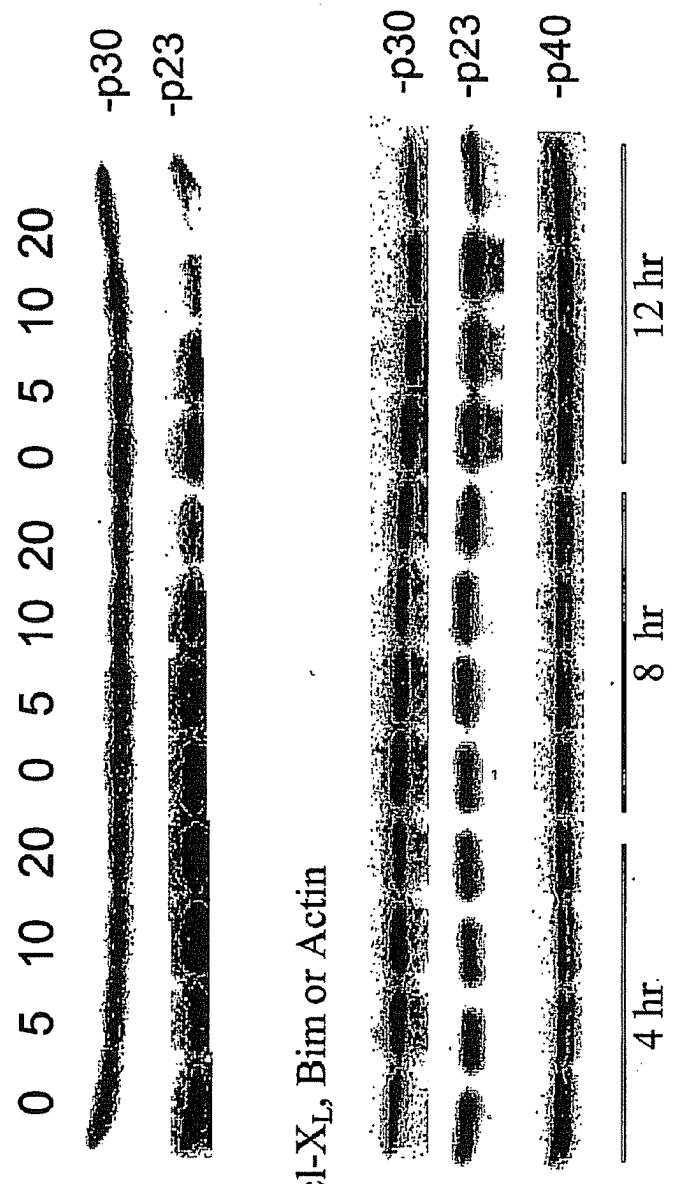

IP: Bcl-XL and WB: Bcl-XL or Bim
Gossypol μM- 0 5 10 20 0 5 10 20 0 5 10 20
Bcl-XL-
-p30
Bim-
-p23
WB Only: Bcl-XL, Bim or Actin
Bcl-XL-
-p30
Bim-
-p23
Actin-
-p40
4 hr
8 hr
12 hr

Figure 45

Competitive binding curve of apogossypol against Bcl-2

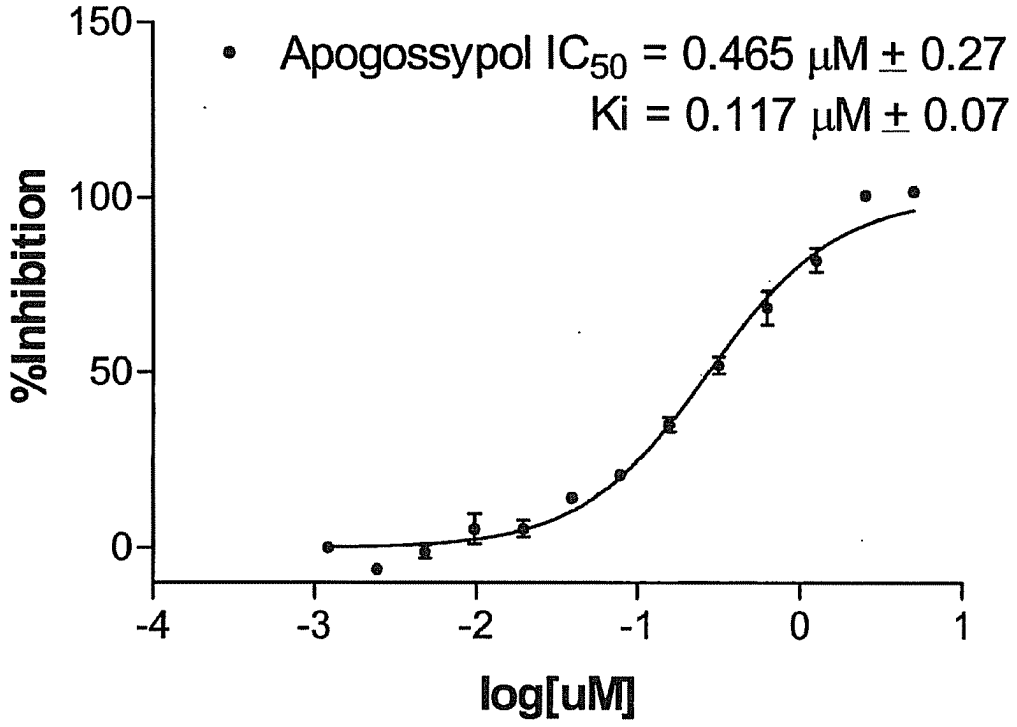
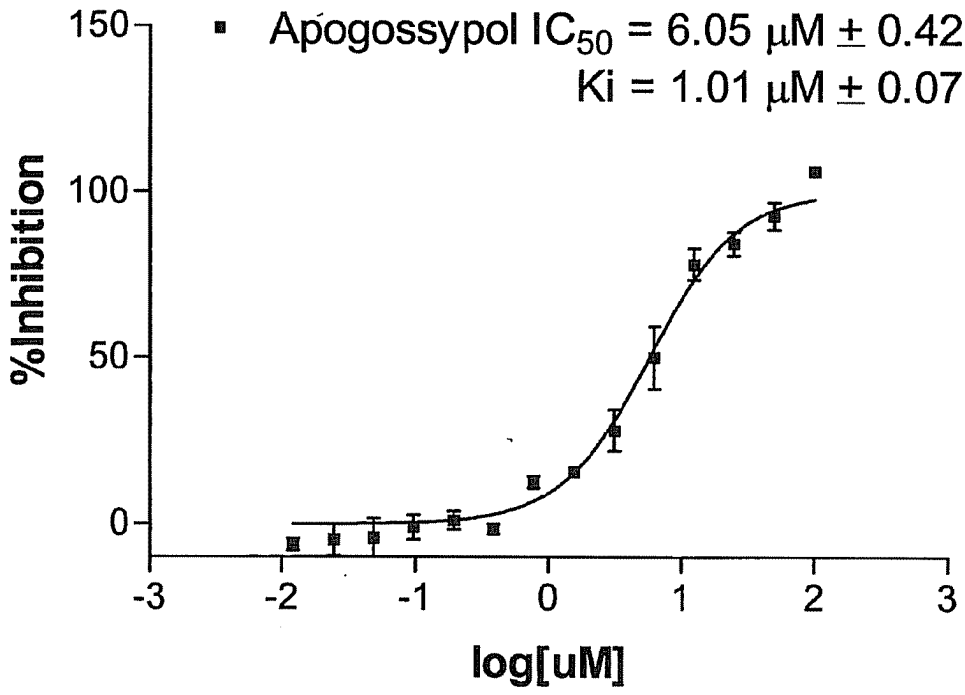


Figure 46

Competitive binding curve of apogossypol against Bcl-X_L.



SMALL MOLECULE ANTAGONISTS OF BCL-2 FAMILY PROTEINS

[0001] This application is a continuation in part of U.S. patent application Ser. No.10/158,769 filed May 30, 2002, and PCT/US02/17206 filed May 30, 2002, both of which claim priority to U.S. Provisional Patent Application Serial No. 60/293,983, filed May 30, 2001, the contents of each of which are fully incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to naturally occurring and chemically synthesized small molecule antagonists of Bcl-2 family proteins. In particular, the present invention provides gossypol compounds (e.g., isomers, enantiomers, racemic compounds, metabolites, derivatives, pharmaceutically acceptable salts, in combination with acids or bases, and the like) and methods of using these compounds as antagonists of the anti-apoptotic effects of Bcl-2 family member proteins (e.g., Bcl-2, Bcl-X_L, and the like). The present invention also provides compositions comprising gossypol compounds and optionally one or more additional therapeutic agents (e.g., anticancer/chemotherapeutic agents). The present invention also provides methods for treating diseases and pathologies (e.g., neoplastic diseases) comprising administering a composition comprising gossypol compounds and optionally one or more additional therapeutic agents (e.g., anticancer/chemotherapeutic agents) and/or techniques (e.g., radiation therapies, surgical interventions, and the like) to a subject or in vitro cells, tissues, and organs.

BACKGROUND OF THE INVENTION

[0003] Multicellular organisms use a process called apoptosis to instruct damaged or unnecessary cells to destroy themselves for the good of the organism. Control of the apoptotic process is very important for the normal development of the organism. For example, fetal development of fingers and toes requires the controlled removal, by apoptosis, of excess interconnecting tissues, as does proper formation of neural synapses within the brain. Careful control of apoptosis is also important to adult organisms, for instance, controlled apoptosis is responsible for the sloughing of the inner lining of the uterus (the endometrium) at the start of menstruation.

[0004] Apoptosis not only plays an important role in tissue sculpting during fetal development and normal cellular maintenance, it is also the primary defense against rogue cells that threaten the well being of the entire organism. For instance, in the cell mediated immune response, effector cells (e.g., cytotoxic T lymphocytes "CTLs") destroy virus-infected host cells by inducing the infected host cells to undergo apoptosis. The organism subsequently relies in turn upon the apoptotic process to destroy the effector cells when they are no longer needed. Autoimmunity is prevented by the CTLs inducing apoptosis in each other and even in themselves. Defects in this process are associated with a variety of autoimmune diseases such as lupus erythematosus and rheumatoid arthritis.

[0005] Multicellular organisms use the apoptotic process to instruct cells with damaged nucleic acids (e.g., DNA) to destroy themselves prior to becoming cancerous. However, some cancer-causing viruses prevent apoptosis in transformed cells. For example, several human papilloma viruses (HPVs) are implicated in causing cervical cancer by suppressing apoptotic removal of transformed cells through the production of a protein, E6, which inactivates the p53 apoptosis promoter. Epstein-Barr virus (EBV), the causative agent of mononucleosis and Burkitt's lymphoma, a solid tumor of B lymphocytes, produces a first protein similar to Bcl-2, and a second that causes transformed cells to increase production of Bcl-2. The expression of various Bcl-2 family proteins helps virus-transformed cells resist apoptosis. Still other viruses manipulate the cell's apoptotic machinery without directly resulting in the development of a cancer. For example, destruction of the immune system in individuals infected with the human immunodeficiency virus (HIV) is thought to progress through infected CD4+ T cells (about 1 in 100,000) instructing their sister cells to undergo apoptosis. Faulty regulation of the apoptotic machinery has also been implicated in various degenerative conditions and vascular diseases.

[0006] Some cancers that arise by non-viral means have also developed mechanisms to escape destruction by apoptosis. Melanoma cells, for instance, avoid apoptosis by inhibiting the expression of the gene encoding the apoptosis effector protein Apaf-1. Other cancers, especially lung and colon, secrete elevated levels of soluble decoy molecules that bind FasL, inhibiting it from binding to Fas. CTLs are thus prohibited from destroying these cancer cells. Other cancer cells express high levels of FasL, again, avoiding destruction by the CTLs.

[0007] It is apparent that the controlled regulation of the apoptotic process and the apoptotic machinery is vital to the survival of multicellular organisms. Typically, the biochemical changes that occur in a cell instructed to undergo apoptosis occur in an orderly procession. However, as shown above, flawed regulation of these process can cause serious harm.

[0008] There have been various attempts to use small molecules to control and restore regulation of the apoptotic machinery in aberrant cells (e.g., cancer cells). Generally, these attempts have had limited success as treatments for the underlying diseases for a number of reasons, including high toxicity, low bioavailability, high costs, and the like. What is needed are improved methods and compositions for regulating apoptosis in subjects afflicted with diseases and conditions that are characterized by faulty regulation of the apoptotic process.

SUMMARY OF THE INVENTION

[0009] It is generally accepted in the field of molecular oncology that most, if not all, malignant cancer cells harbor (at a minimum) two derangements that lead to the malignant phenotype: a proliferative lesion, causing cells to multiply inappropriately, and an apoptotic lesion, that prevents the cell(s) from executing the apoptosis program in response to either the detection, within the cell, of these genetic abnormalities (e.g., up-regulation of a growth or mitosis oncogene like Ras or Myc), or the pharmacological effects of cell death-inducing cancer therapeutic drugs or radiation therapy. The apoptotic lesion confers on the cells a survival advantage in the face of either further accumulated oncogenic lesions, or exposure to pharmacologically effective levels of cancer therapeutic drugs or radiation therapy.

[0010] A number of apoptotic lesions have been described in tumor cells (e.g. loss of p53, decreased Apaf-1, increased IAPs, decreased caspases), both in vitro and in vivo, most notably enhanced expression and accumulation of proteins of the anti-apoptotic Bcl-2 gene family. Bcl-2 is the prototypical member of this family, which includes Bcl-X_L, Mcl-1, A1, and Boo/Diva proteins. Bcl-2 is a human oncogene that prevents the activation of the apoptosis program in many cells, and when expressed at inappropriately high levels in cancerous or pre-cancerous cells, confers on them a selective advantage. Bcl-2 and Bcl-X_L are overexpressed in many types of human cancer (e.g., breast, prostate, colorectal, lung, etc.), including Non-Hodgkin's lymphoma, which is caused by a chromosomal translocation (t14, 18) that leads to overexpression of Bcl-2, suggesting that many cancer cell types depend on the elevated levels of Bcl-2 and/or Bcl-X_L to survive the other cellular derangements that simultaneously both define them as cancerous or pre-cancerous cells and cause them to attempt to execute the apoptosis pathway. Also, increased expression of Bcl-2 family proteins has been recognized as a basis for the development of resistance to cancer therapeutic drugs and radiation that act in various ways to induce cell death in tumor cells.

[0011] The induction of apoptosis in cancer cells or their supporting cells (e.g., neovascular cells in the tumor vasculature) is thought to be a universal mechanism of action for virtually all of the effective cancer therapeutic drugs or radiation therapies on the market or in practice today. The present invention contemplates that exposure of humans suffering from cancer to therapeutically effective amounts of drug(s) (e.g., small molecules) that inhibit the function(s) of Bcl-2 and Bcl-X_L kills cancer cells or supporting cells outright (those cells whose continued survival is dependent on the overactivity of Bcl-2 or Bcl-X_L) or to render such cells as a population more susceptible to the cell death-inducing activity of cancer therapeutic drugs or radiation therapies. The present invention contemplates that inhibitors of Bcl-2/Bcl-X_L satisify an unmet need for the treatment of multiple cancer types, either when administered as monotherapy to induce apoptosis in cancer cells dependent on Bcl-2/Bcl-X_L function, or when administered in a temporal relationship with other cell death-inducing cancer therapeutic drugs or radiation therapies so as to render a greater proportion of the cancer or supportive cells vulnerable to executing the apoptosis program compared to the corresponding proportion of cells in a subject treated only with the cancer therapeutic drug or radiation therapy alone.

[0012] During the course of the development of the present invention, gossypol was found to bind to a key binding site (the BH3-binding site) in both Bcl-2 and Bcl-X_L, to which the natural protein antagonists of Bcl-2/Bcl-X_L, including Bax, Bak, Bad, Bim, NOXA, and PUMA bind. Thus, particularly preferred embodiments provide compositions and methods comprising gossypol compounds (e.g., (−)-gossypol, (−)-gossypol acetic acid, and the like) having Bcl-2/B Bcl-X_L inhibitory activity, and that cause cells that depend for their survival, at least in part, on Bcl-2 and/or Bcl-X_L to execute the apoptosis program and die. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice (make and use) the present invention. Nonetheless, it is contemplated that two classes of such Bcl-2/Bcl-X-dependent cells are 1) a first class of cells that are internally deranged to such an extent that the "flux" through the apoptosis pathway would be sufficient, were it not for the elevated levels of Bcl-2 and/or Bcl-X_L, to trigger execution of the apoptosis program; and 2) a second class of cells whose apoptosis program has been stimulated in response to a cancer therapeutic drug or radiation but below a threshold that has been set in that cell by the elevated levels of Bcl-2/Bcl-X_L. Either class of cells, by virtue of being dependent on Bcl-2, Bcl-X or both for their survival, can be killed by an effective amount of a Bcl-2/Bcl-X_L inhibiting compound (e.g., (−)-gossypol, (−)-gossypol acetic acid, and the like).

[0013] Indeed, gossypol compounds (e.g., (−)-gossypol) can induce the death of tumor cells in vitro and can reduce tumor burden in mice bearing human tumor xenografts (See, Examples). In addition, gossypol compounds (e.g., (−)-gossypol), by virtue of reducing the activity of Bcl-2 and/or Bcl-X_L in cancer cells or supporting cells, increases the proportion of cells in a subject that will respond to the cell-damaging effects of cancer therapeutic drugs or radiation therapy by executing the apoptosis program, leading to a greater tumor response in subjects treated in combination with gossypol and the cancer therapeutic drug or radiation therapy compared to those treated with chemo/radiation alone. This enhanced tumor response will be reflected in any of a number of clinically desirable endpoints, including tumor shrinkage and/or loss, time to tumor progression (TTP), or survival. In additional preferred embodiments, gossypol compounds (e.g., (−)-gossypol), in combination with any of a number of cancer therapeutic drugs or radiation, produces added tumor reductions over chemo/radiation alone (See, Examples). In some examples, gossypol compounds (e.g., (−)-gossypol) produce "synergistic" apoptosis (in vitro isobologram Examples) or tumor responses (in vivo Examples). The in vivo synergism even leads, in some cases, to regression of tumors that would not regress with either agent alone.

[0014] From these observations, combination treatment of human subjects with a therapeutically effective amount of a gossypol compound (e.g., (−)-gossypol) and an approved course of cancer therapeutic drugs or radiation, produces a greater tumor response and clinical benefit in such subjects compared to those treated with gossypol compound or cancer drugs/radiation alone. It is contemplated that gossypol (e.g., (−)-gossypol) acts either to kill cells outright or to increase the proportion of cancer or supporting cells that respond to the apoptosis-inducing effects of drugs/radiation by executing the apoptosis program. Put another way, because gossypol compounds lower the apoptotic threshold of all cells that express Bcl-2 and/or Bcl-X_L, the proportion of cells that successfully execute the apoptosis program in response to the apoptosis-inducing activity of cancer drugs/radiation is increased. Alternatively, gossypol compounds can be used to allow administration of a lower, and therefore less toxic and more tolerable, dose of a cancer therapeutic drug or radiation to produce the same tumor response/clinical benefit as the conventional dose of the drug/radiation alone. Since the doses for all approved cancer drugs and radiation treatments are known, the present invention contemplates the various combinations of them with gossypol compounds. Also, since gossypol compounds act at least in part by inhibiting Bcl-2 and/or Bcl-X_L, the exposure of cancer and supporting cells to a therapeutically effective amount of gossypol can be temporally linked to coincide

with the attempts of cells to execute the apoptosis program in response to the cancer drug or radiation therapy. Thus, in some embodiments, administering the compositions and methods of the present invention in view of certain temporal relationships, which can be tested in clinical trials, provides especially efficacious therapeutic practices.

[0015] The present invention relates to naturally occurring and chemically synthesized small molecule antagonists of Bcl-2 family proteins. In particular, the present invention provides gossypol compounds (e.g., isomers, enantiomers, racemic compounds, metabolites, derivatives, pharmaceutically acceptable salts, in combination with acids or bases, and the like) and methods of using these compounds as antagonists of the anti-apoptotic effects of Bcl-2 family member proteins (e.g., Bcl-2, Bcl-X_L, and the like). The present invention also provides compositions comprising gossypol compounds and optionally one or more additional therapeutic agents (e.g., anticancer/chemotherapeutic agents). The present invention also provides methods for treating diseases and pathologies (e.g., neoplastic diseases) comprising administering a composition comprising gossypol compounds and optionally one or more additional therapeutic agents (e.g., anticancer/chemotherapeutic agents) and/or techniques (e.g., radiation therapies, surgical interventions, and the like) to a subject or in vitro cells, tissues, and organs.

[0016] The term cancer is generally used to described hundreds of neoplastic diseases and neoplasias. The neoplastic growths can be benign or malignant. There are three broad types of cancer: carcinomas, sarcomas, and hematologic malignancies (more commonly known as lymphomas and leukemias). Each type of cancer can affect almost any organ or part of the body. Carcinomas originate in the outer layer of cells of the skin and internal membranes (e.g., breasts, lungs, intestines, skin, prostate, etc.). Sarcomas arise from connective tissue such as bone, muscle, cartilage and blood vessels. Lymphomas and leukemias, hematologic cancers, arise in the blood or blood-forming organs such as the spleen, lymph nodes and bone marrow.

[0017] Cancer cells include tumor cells, neoplastic cells, malignant cells, metastatic cells, and hyperplastic cells. Neoplastic cells can be benign or malignant. Neoplastic cells are benign if they do not invade or metastasize. A malignant cell is one that is able to invade and/or metastasize. Hyperplasia is a pathologic accumulation of cells in a tissue or organ, without significant alteration in structure or function.

[0018] Malignant tumors are generally referred to as being either primary or secondary. Primary tumors arise directly in the tissue in which they are found. Secondary tumors, or metastases, are tumors that originated elsewhere in the body, but have now spread, to a distant tissues and organs. There are some malignancies that are predisposed to spreading to the skeleton. Prostrate cancer and breast carcinoma typically metastasize to bone. Another frequent site of tumor metastasis is the brain.

[0019] The common routes for tumor metastasis are direct growth into adjacent structures, spread through the vascular or lymphatic systems, and tracking along tissue planes and body spaces (e.g., peritoneal fluid, cerebrospinal fluid, etc.). Clinically, most patients die from metastatic disease.

[0020] The present invention is not limited to any particular mechanism. Indeed, an understanding of any particular mechanism is unnecessary to practice (make and use) the compositions and methods of the present invention. Nonetheless, it is believed that the molecular mechanisms involved in metastatic tumor maintenance are different from those involved in primary tumor maintenance. The present invention contemplates that elucidation of the cellular mechanisms associated with metastatic cancer maintenance and metastasis provides insight into the development of new effective anticancer treatments.

[0021] Malignant tumor progression, in many cases, is correlated with increased migratory capacity involving, at least in part, altered metalloproteolytic activity. Tumor invasion is thought to rely on the modification of cell adhesion and the proteolysis of extracellular matrix components. Bcl-2 is though to have specific effects on the molecules involved in cancer cell migration and invasion (See, V. Amberger, et al., Cancer Res., 58:149-158 (1998)). Cancer cells that express Bcl-2 proteins may be more invasive than other cancer cells. Bcl-2 proteins are also thought to enhance cancer cell migration and invasion by altering the expression of metalloproteinases and their inhibitors. Wick et al. (W. Wick, et al., FEBS Lett., 440:419-424 (1998)) reported that ectopic expression of Bcl-2 in two glioma cell lines significantly enhanced migration and invasion in a Matrigel-coated membrane invasion assay (See, S. Mohanam, et al., Cancer Res. 53:4143-4147 (1993)) and a fetal rat brain confrontation assay (See, P. Pedersen, et al., Cancer Res., 53:5158-5165 (1993)). Bcl-2 expression is also thought to lead to activation and/or increase of matrix metalloproteinases (e.g., MMP-2, MMP-9) or the cell surface urokinase-type plasminogen activator (u-PA), and reductions of metalloproteinases tissue inhibitors (TIMPs).

[0022] Successful migration and invasion of cancer cells requires the ability to survive, or to become resistant to, the endogenous apoptotic death program signals once the cancer cell has detached from the primary tumor tissue. The present invention is not limited to any particular mechanism. Indeed, an understanding of any particular mechanism is unnecessary to practice (make and use) the compositions and methods of the present invention. Nonetheless, the present invention contemplates that overexpression of anti-apoptotic Bcl-2 proteins provides tumor cells with a mechanism for surviving in new and non-permissive environments (e.g., metastatic sites), and contributes to the organospecific pattern of clinical metastatic cancer spread. It is further contemplated that overexpression of Bcl-X_L counteracts the proapoptotic signals in the cancer cells' microenvironment, thus favoring successful development of metastases. The bcl-X_L gene is further thought to play a role in breast cancer dormancy by promoting the survival of cells in metastatic foci in specific organs (See, Nuria Rubio, Lab Invest, 81:725-734 (2001)). For example, in human breast carcinomas, the overexpression of anti-apoptotic Bcl-X_L protein is thought to increase metastatic potential by providing, at least in part, increased resistance to cytokines, overriding apoptotic signals, enhancing anchorage-independent growth (e.g., caused by a modified interaction with the extracellular matrix), and increasing cell survival in the circulation (Fernandez et al., Cell Death Differ., 7:350-359 (2000)). It has been shown that a number of cell adhesion molecules play a role in metastasis and that integrins are especially involved in tumorigenic spread. Integrins are implicated in cell-cell and cell-extracellular matrix (ECM) interactions, signaling, sensing cellular microenvironment, and in mod-

erating cellular activities including, but not limited to, migration, differentiation, survival and tissue (re)modeling in both normal and pathological states. The present invention contemplates that anti-apoptotic proteins such as Bcl-2 and/or Bcl-X_L regulate cell-cell interactions (See, J. Reed, Nature, 387:773-776 (1997)). Down-regulation of cell surface integrins by antibodies could lead to induction of apoptosis. For example, Bcl-2 expression is up-regulated by $\alpha_5\beta_1$ integrins preventing apoptosis when cells are detached from the matrix (See, S. Frisch and E. Ruoslahti, Curr. Opin. Cell Biol., 9:701-706 ((1997)). Expression of Bcl-2 is contemplated to promote the metastatic potential of the human breast cancer cell line MCF7 in vivo and migratory and invasive properties in vitro (See, D. Del Bufalo, et al., FASEB J., 11:947-953 (1997)).

[0023] In some embodiments, the present invention provides methods of inhibiting tumor metastasis in a subject, comprising administering to the subject a gossypol compound (e.g., (−)-gossypol) that decreases the survival of metastatic cells by inhibiting cellular activity of Bcl-2/Bcl-X_L proteins. In certain other embodiments, the present invention provides methods of treating (e.g., ameliorating and/or preventing) cancer metastasis comprising administering to a subject having a cancer metastasis a therapeutically effective amount of a gossypol compound (e.g., (−)-gossypol), and optionally one or more anticancer and/or anti-neoplastic agents. The present invention is not intended to be limited to administering any particular gossypol compound, or compounds for the prevention (or retarding) of tumor metastasis. Indeed, a number of gossypol compounds are contemplated as being useful in the preventing, attenuating, or retarding of tumor metastasis including, but not limited to, (±)-gossypol; (−)-gossypol; (+)-gossypol; (±)-gossypolone; (−)-gossypolone; (+)-gossypolone; (±)-gossypol acetic acid; (−)-gossypol acetic acid; (+)-gossypol acetic acid; (±)-ethyl gossypol; (−)-ethyl gossypol; (+)-ethyl gossypol; (±)-hemigossypolone; (−)-hemigossypolone; (+)-hemigossypolone; Schiff's base of (±)-gossypol; Schiff's base of (−)-gossypol; Schiff's base of (+)-gossypol; Schiff's base of (+)-gossypolone; Schiff's base of (−)-gossypolone; Schiff's base of (+)-gossypolone; Schiff's base of (+)-gossypol acetic acid; Schiff's base of (−)-gossypol acetic acid; Schiff's base of (+)-gossypol acetic acid; Schiff's base of (+)-ethyl gossypol; Schiff's base of (−)-ethyl gossypol; Schiff's base of (+)-ethyl gossypol; Schiff's base of (+)-hemigossypolone; Schiff's base of (−)-hemigossypolone; Schiff's base of (+)-hemigossypolone, (±)-apogossypol, (−)-apogossypol, (+)-apogossypol, (±)-apogossypol acetic acid, (−)-apogossypol acetic acid, (+)-apogossypol acetic acid, (±)-ethyl apogossypol, (−)-ethyl apogossypol, (+)-ethyl apogossypol, and the like. The present invention further contemplates that a range of additional (second) chemotherapeutic, anticancer, or anti-neoplastic agents, radiation therapies, and/or surgical interventions can optionally be combined (in any temporal order) with gossypol compounds to prevent or retard tumor metastasis in a subject. In this regard, the present invention describes various exemplary additional (second) agents and therapies that are useful in certain embodiments of the present invention directed to tumor metastasis.

[0024] An important goal in oncology is to optimize the use of available treatment options (e.g., chemotherapy, radiation therapy, surgery, and the like) to achieve maximum obtainable therapeutic effect while preserving organs and the subject's general quality of life.

[0025] Bcl-2 is the founding member of a family of proteins and was first isolated as the product of an oncogene. The Bcl-2 family of proteins now includes both anti-apoptotic molecules such as Bcl-2 and Bcl-X_L and pro-apoptotic molecules such as Bax, Bak, Bid, and Bad. Bcl-2 and Bcl-X_L are thought to be important regulators of Bcl-2 family mediated apoptosis.

[0026] In preferred embodiments, the administration of gossypol compounds is contemplated to provide an effective treatment of neoplastic conditions and other disorders that involve either the aberrant hyperproliferation or defective apoptosis of cells (e.g., tumor cells).

[0027] In other preferred embodiments, the present invention provides methods of treatment or prophylaxis of cancers in a subject comprising administering to the subject a gossypol compound in an amount effective to inhibit Bcl-2 and/or Bcl-X_L, thus inducing apoptosis and suppressing tumor growth and/or proliferation. Preferably, a gossypol compound is administered in conjunction with another agent or treatment, such as a chemotherapeutic agent (e.g., a tumor cell apoptosis promoting agent) or radiation. The present invention is not limited to any particular mechanism. Indeed, an understanding of any particular mechanism is unnecessary to practice (make and use) the methods and compositions of the present invention. Nonetheless, it is contemplated that increasing apoptosis in target cells (e.g., pathogenic cells including, but not limited to, cancer cells) reestablishes normal apoptotic control associated with basal expression of Bcl-2 and/or Bcl-X_L and/or another anti-apoptotic Bcl-2 family protein (e.g. Bcl-w).

[0028] The methods of the present invention are particularly well suited for the treatment of cancers characterized by overexpression of Bcl-2 family proteins including, but not limited to, Bcl-2 and/or Bcl-X_L.

[0029] In some preferred embodiments, the methods of the present invention provide effective amounts of gossypolone to a patient having a condition characterized by the overexpression of Bcl-2 family proteins, and optionally one or more anticancer or anti-neoplastic agent including, but not limited to radiation therapy.

[0030] In one preferred embodiment, the present invention provides a method of modulating apoptosis in a cell comprising: providing a cell, wherein the cell overexpresses a Bcl-2 family protein; a gossypol compound; and treating the cell with an effective amount of the gossypol compound under conditions such that apoptosis in the cell is modulated.

[0031] The methods of the present invention are not intended to be limited to administration of any particular gossypol compounds. Indeed, the present invention contemplates the administration of a number of gossypol enantiomers, metabolites, derivatives, and pharmaceutically acceptable salts, as well as Schiff's bases of these compounds. For example, gossypol compounds suitable for use in the present invention include, but are not limited to, (±)-gossypol; (−)-gossypol; (+)-gossypol; (±)-gossypolone; (−)-gossypolone; (+)-gossypolone; (±)-gossypol acetic acid; (−)-gossypol acetic acid; (+)-gossypol acetic acid; (+)-ethyl gossypol; (−)-ethyl gossypol; (+)-ethyl gossypol; (±)-hemigossypolone; (−)-hemigossypolone; (+)-hemigossypolone;

Schiff's base of (±)-gossypol; Schiff's base of (−)-gossypol; Schiff's base of (+)-gossypol; Schiff's base of (±)-gossypolone; Schiff's base of (−)-gossypolone; Schiff's base of (+)-gossypolone; Schiff's base of (±)-gossypol acetic acid; Schiff's base of (−)-gossypol acetic acid; Schiff's base of (+)-gossypol acetic acid; Schiff's base of (±)-ethyl gossypol; Schiff's base of (−)-ethyl gossypol; Schiff's base of (+)-ethyl gossypol; Schiff's base of (±)-hemigossypolone; Schiff's base of (−)-hemigossypolone; Schiff's base of (+)-hemigossypolone, (±)-apogossypol, (−)-apogossypol, (+)-apogossypol, (±)-apogossypol acetic acid, (−)-apogossypol acetic acid, (+)-apogossypol acetic acid, (±)-ethyl apogossypol, (−)-ethyl apogossypol, (+)-ethyl apogossypol, and the like.

[0032] In preferred embodiments, the present invention provides administering the (−)-gossypol enantiomer to a patient having a condition characterized by overexpression of a Bcl-2 family protein. In some embodiments, the overexpressed Bcl-2 family proteins contemplated include, but are not limited to, Bcl-2, Bcl-X_L, Mcl-1, Bcl-w, A1/BFL-1, BOO-DIVA, Bcl-6, Bcl-8, and Bcl-y. In still some other embodiments, the overexpressed Bcl-2 family proteins have pro-apoptotic activity. In yet other embodiments, the overexpressed Bcl-2 family proteins have anti-apoptotic activity.

[0033] In some embodiments, the compositions and methods of the present invention are used to treat diseased cells, tissues, organs, or pathological conditions and/or disease states in a subject organism (e.g., a mammalian subject including, but not limited to, humans and veterinary animals), or in in vitro and/or ex vivo cells, tissues, and organs. In this regard, various diseases and pathologies are amenable to treatment or prophylaxis using the present methods and compositions. A non-limiting exemplary list of these diseases and conditions includes, but is not limited to, breast cancer, prostate cancer, lymphoma, skin cancer, pancreatic cancer, colon cancer, melanoma, malignant melanoma, ovarian cancer, brain cancer, primary brain carcinoma, head-neck cancer, glioma, glioblastoma, liver cancer, bladder cancer, non-small cell lung cancer, head or neck carcinoma, breast carcinoma, ovarian carcinoma, lung carcinoma, small-cell lung carcinoma, Wilms' tumor, cervical carcinoma, testicular carcinoma, bladder carcinoma, pancreatic carcinoma, stomach carcinoma, colon carcinoma, prostatic carcinoma, genitourinary carcinoma, thyroid carcinoma, esophageal carcinoma, myeloma, multiple myeloma, adrenal carcinoma, renal cell carcinoma, endometrial carcinoma, adrenal cortex carcinoma, malignant pancreatic insulinoma, malignant carcinoid carcinoma, choriocarcinoma, mycosis fungoides, malignant hypercalcemia, cervical hyperplasia, leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, chronic granulocytic leukemia, acute granulocytic leukemia, hairy cell leukemia, neuroblastoma, rhabdomyosarcoma, Kaposi's sarcoma, polycythemia vera, essential thrombocytosis, Hodgkin's disease, non-Hodgkin's lymphoma, soft-tissue sarcoma, osteogenic sarcoma, primary macroglobulinemia, and retinoblastoma, and the like, T and B cell mediated autoimmune diseases; inflammatory diseases; infections; hyperproliferative diseases; AIDS; degenerative conditions, vascular diseases, and the like. In some embodiments, the cancer cells being treated are metastatic.

[0034] In some embodiments, infections suitable for treatment with the compositions and methods of the present invention include, but are not limited to, infections caused by viruses, bacteria, fungi, mycoplasma, prions, and the like. The present invention is not intended to be limited, however, to treating of any particular infections or infectious agents.

[0035] In one preferred embodiment, the present invention provides methods of modulating cell division in a tissue comprising: providing a tissue, wherein the tissue overexpresses a Bcl-2 protein; a gossypol compound; an anticancer agent; and treating the tissue with effective amounts of the gossypol compound and the anticancer agent under conditions such that cell division is modulated. In some of these embodiments, the present invention contemplates gossypol compounds bind to Bcl-2 family proteins thus modulating cell division. In still further embodiments, the methods optionally comprise one or more antineoplastic and/or anti-hyperproliferative chemotherapeutic agents (e.g., small or large molecule drugs, polypeptides, polynucleotides, synthetic or naturally occurring chemical compounds, and the like), or therapies (e.g., radiation therapy, surgical interventions, etc.).

[0036] In yet another embodiment, the present invention provides methods of treating a subject (e.g., a patient) comprising administering a gossypol compound to a subject overexpressing a Bcl-2 family protein. In a preferred example of these embodiments, the gossypol compound binds to a Bcl-2 family protein.

[0037] Some embodiments of the present invention are directed to providing methods of treating a subject comprising administering a gossypol compound and one or more anticancer agents to a subject overexpressing a Bcl-2 family protein.

[0038] A number of suitable anticancer agents are contemplated for use in the methods of the present invention. Indeed, the present invention contemplates, but is not limited to, administration of numerous anticancer agents such as: agents that induce apoptosis; polynucleotides (e.g., antisense, ribozymes, siRNA); polypeptides (e.g., enzymes and antibodies); biological mimetics (e.g., gossypol or BH3 mimetics); agents that bind (e.g., oligomerize or complex) with a Bcl-2 family protein such as Bax; alkaloids; alkylating agents; antitumor antibiotics; antimetabolites; hormones; platinum compounds; monoclonal or polyclonal antibodies (e.g., antibodies conjugated with anticancer drugs, toxins, defensins, etc.), toxins, radionuclides; biological response modifiers (e.g., interferons (e.g., IFN-α, etc.) and interleukins (e.g., IL-2, etc.), etc.); adoptive immunotherapy agents; hematopoietic growth factors; agents that induce tumor cell differentiation (e.g., all-trans-retinoic acid, etc.); gene therapy reagents (e.g., antisense therapy reagents and nucleotides); tumor vaccines; angiogenesis inhibitors; proteosome inhibitors: NF kappa β modulators; anti-CDK compounds; HDAC inhibitors; and the like. Numerous other examples of chemotherapeutic compounds and anticancer therapies suitable for co-administration with the disclosed gossypol compounds are known to those skilled in the art.

[0039] In preferred embodiments, anticancer agents comprise agents that induce or stimulate apoptosis. Agents that induce apoptosis include, but are not limited to, radiation (e.g., X-rays, gamma rays, UV); kinase inhibitors (e.g., Epidermal Growth Factor Receptor (EGFR) kinase inhibitor, Vascular Growth Factor Receptor (VGFR) kinase inhibitor, Fibroblast Growth Factor Receptor (FGFR) kinase

inhibitor, Platelet-derived Growth Factor Receptor (PDGFR) kinase inhibitor, and Bcr-Abl kinase inhibitors such as GLEEVEC); antisense molecules; antibodies (e.g., HERCEPTIN, RITUXAN, ZEVALIN, and AVASTIN); anti-estrogens (e.g., raloxifene and tamoxifen); anti-androgens (e.g., flutamide, bicalutamide, finasteride, aminoglutethamide, ketoconazole, and corticosteroids); cyclooxygenase 2 (COX-2) inhibitors (e.g., celecoxib, meloxicam, NS-398, and non-steroidal anti-inflammatory drugs (NSAIDs)); anti-inflammatory drugs (e.g., butazolidin, DECADRON, DELTASONE, dexamethasone, dexamethasone intensol, DEXONE, HEXADROL, hydroxychloroquine, METICORTEN, ORADEXON, ORASONE, oxyphenbutazone, PEDIAPRED, phenylbutazone, PLAQUENIL, prednisolone, prednisone, PRELONE, and TANDEARIL); and cancer chemotherapeutic drugs (e.g., irinotecan (CAMPTOSAR), CPT-11, fludarabine (FLUDARA), dacarbazine (DTIC), dexamethasone, mitoxantrone, MYLOTARG, VP-16, cisplatin, carboplatin, oxaliplatin, 5-FU, doxorubicin, gemcitabine, bortezomib, gefitinib, bevacizumab, TAXOTERE or TAXOL); cellular signaling molecules; ceramides and cytokines; and staurosporine, and the like.

[0040] In still other embodiments, the compositions and methods of the present invention provide gossypol compounds and at least one anti-hyperproliferative or antineoplastic agent(s) selected from alkylating agents, antimetabolites, and natural products (e.g., herbs and other plant and/or animal derived compounds).

[0041] Alkylating agents suitable for use in the present compositions and methods include, but are not limited to: 1) nitrogen mustards (e.g., mechlorethamine, cyclophosphamide, ifosfamide, melphalan (L-sarcolysin); and chlorambucil); 2) ethylenimines and methylmelamines (e.g., hexamethylmelamine and thiotepa); 3) alkyl sulfonates (e.g., busulfan); 4) nitrosoureas (e.g., carmustine (BCNU); lomustine (CCNU); semustine (methyl-CCNU); and streptozocin (streptozotocin)); and 5) triazenes (e.g., dacarbazine (DTIC; dimethyltriazenoimidazolecarboxamide).

[0042] In some embodiments, antimetabolites suitable for use in the present compositions and methods include, but are not limited to: 1) folic acid analogs (e.g., methotrexate (amethopterin)); 2) pyrimidine analogs (e.g., fluorouracil (5-fluorouracil; 5-FU), floxuridine (fluorode-oxyuridine; FudR), and cytarabine (cytosine arabinoside)); and 3) purine analogs (e.g., mercaptopurine (6-mercaptopurine; 6-MP), thioguanine (6-thioguanine; TG), and pentostatin (2'-deoxycoformycin)).

[0043] In still further embodiments, chemotherapeutic agents suitable for use in the compositions and methods of the present invention include, but are not limited to: 1) vinca alkaloids (e.g., vinblastine (VLB), vincristine); 2) epipodophyllotoxins (e.g., etoposide and teniposide); 3) antibiotics (e.g., dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin), and mitomycin (mitomycin C)); 4) enzymes (e.g., L-asparaginase); 5) biological response modifiers (e.g., interferon-alfa); 6) platinum coordinating complexes (e.g., cisplatin (cis-DDP) and carboplatin); 7) anthracenediones (e.g., mitoxantrone); 8) substituted ureas (e.g., hydroxyurea); 9) methylhydrazine derivatives (e.g., procarbazine (N-methylhydrazine; MIH)); 10) adrenocortical suppressants (e.g., mitotane (o,p'-DDD) and aminoglutethimide); 11) adrenocorticosteroids (e.g., prednisone); 12) progestins (e.g., hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate); 13) estrogens (e.g., diethylstilbestrol and ethinyl estradiol); 14) antiestrogens (e.g., tamoxifen); 15) androgens (e.g., testosterone propionate and fluoxymesterone); 16) antiandrogens (e.g., flutamide): and 17) gonadotropin-releasing hormone analogs (e.g., leuprolide).

[0044] In still other embodiments, the present invention provides methods of treating cancer in a subject comprising administering to a patient having a condition characterized by overexpression of a Bcl-2 family protein an effective amount of a gossypol compound.

[0045] Additional embodiments are directed to methods of treating cancer in a subject comprising administering to a subject having cancer, wherein the cancer is characterized by overexpression of a Bcl-2 family protein, an effective amount of a gossypol compound and one or more anticancer agents.

[0046] Still other methods are directed to treating cancer in a subject comprising administering to a patient having cancer, wherein the cancer is characterized by resistance to cancer therapies (e.g., chemoresistant, radiation resistant, hormone resistant, and the like), an effective amount of a gossypol compound.

[0047] In some embodiments, the present invention provides methods of treating cancer in a subject comprising administering to a patient having cancer, wherein the cancer is characterized by overexpression of a Bcl-2 family protein, a dose of a gossypol compound sufficient to reduce the overexpression of the Bcl-2 protein.

[0048] In some embodiments of the present invention, methods of treating cancer in a subject comprising administering to a patient having cancer, wherein the cancer is characterized by overexpression of a Bcl-2 family protein, a dose of a gossypol compound and one or more anticancer agents sufficient to reduce the overexpression of the Bcl-2 protein are described.

[0049] In still some other embodiments, the present invention provides methods of treating a hyperproliferative disease, wherein the hyperproliferative disease is characterized by the overexpression of an anti-apoptotic Bcl-2 family protein (e.g., Bcl-2 or Bcl-X_L), in a subject comprising administering to a subject a dose of a gossypol compound sufficient to inhibit the function of the anti-apoptotic Bcl-2 protein and/or to reduce the overexpression of the protein. In some of these embodiments, the methods further comprise administering one or more hyperproliferative and/or antineoplastic therapeutic agents to the subject.

[0050] Some other embodiments of the present invention provide pharmaceutical compositions comprising: a gossypol compound; and instructions for administering the gossypol compound to a subject, the subject characterized by overexpression of a Bcl-2 family protein (e.g., an anti-apoptotic Bcl-2 family member protein). Additional embodiments provide pharmaceutical compositions comprising: a gossypol compound; one or more anticancer agents; and instructions for administering the gossypol compound and the one or more anticancer agents to a subject.

[0051] Further embodiments of the present invention provide pharmaceutical compositions comprising: a gossypol

compound; optionally one or more anticancer agents; and instructions for administering the gossypol compound to a subject, the subject characterized by resistance to a cancer therapy. In preferred embodiments, the instructions included with these kits meet U.S. Food and Drug Administration rules, regulations, and suggestions for the administration, preparation, and distribution of therapeutic kits, compounds, and methods. The instructions optionally also satisfy the domestic regulations placed on therapeutic kits, compounds, and methods, by countries and jurisdictions other than the U.S.

[0052] In yet another embodiment, the present invention provides methods of screening a gossypol compound and a test compound comprising: providing: a gossypol compound; a test compound; a first group of cells; and contacting the first group of cells with the gossypol compound and the test compound; and observing the effects of contacting the first group of cells with the gossypol compound and the test compound. In some of these embodiments, the present invention further provides the additional step of comparing the effects observed in the first cells against a second group of the cells contacted with the gossypol compound alone, or with the test compound alone. Effects that may be observed include, but are not limited to, changes in cell proliferation, changes in apoptotic status, and changes in the expression of Bcl-2 family proteins (e.g., Bcl-2 and/or Bcl-X_L), and the like. In still other embodiments, the present invention further contemplates additional methods for selling test compounds screened/identified by the above methods. In some of these embodiments, test compounds may be offered for sale by a third party in one or more forms (e.g., a kit, including instructions for administering the test compound to a patient). The present invention further provides kits comprising a gossypol compound, one or more chemotherapeutic agents, and instructions for administering the gossypol compound and the chemotherapeutic agents to a subject. In certain of these embodiments, the gossypol compound is (−)-gossypol and the chemotherapeutic agent is selected from docetaxel, TAXOL, cisplatin, and combinations thereof. The present invention is not limited however to kits comprising (−)-gossypol and docetaxel, TAXOL, cisplatin, and combinations thereof.

[0053] The present invention further provides a method of treating or ameliorating a hyperproliferative (or neoplastic) disease in a subject comprising administering to the subject a therapeutically effective dose of a gossypol compound and one or more second agent selected from a chemotherapeutic agent and radiation. In other embodiments, the present invention provides a method of treating or ameliorating a hyperproliferative (or neoplastic) disease in a subject comprising administering to the subject a therapeutically effective dose of a gossypol compound and one or more second agent selected from a chemotherapeutic agent and radiation, with the proviso that a combination of (±)-gossypol, heat, and radiation is not administered. In some embodiments, the one or more second agents comprise anti-neoplastic agents.

[0054] In some methods, a gossypol compound and a chemotherapeutic agent and/or radiation are administered simultaneously. In some other embodiments, a gossypol compound and a chemotherapeutic agent and/or radiation are administered sequentially. In still some other embodiments, a gossypol compound is administered prior to chemotherapeutic agent(s) and/or radiation. In yet other embodiments, a gossypol compound is administered after chemotherapeutic agent(s) and/or radiation.

[0055] The present invention further provides methods, wherein a gossypol compound and a chemotherapeutic agent or radiation are administered with different periodicities, different durations, different concentrations, and/or different administration routes.

[0056] Additional embodiments provide methods wherein a gossypol compound and a chemotherapeutic agent and/or radiation have a synergistic therapeutic effect in a subject or in vitro or ex vivo cells, tissues, or organs.

[0057] In some embodiments, the subject being treated is an animal such as a mammal, fish, or bird. In some embodiments, the mammal being treated is a human. In some other embodiments, the mammal being treated is laboratory animal (e.g., rodent (e.g., mouse, rat, gerbil, rabbit), monkey, dog, pig, cat, etc.). In still some other embodiments, the mammal is a veterinary animal (e.g., dog, cat, horse, cow, pig, goat, sheep, etc.).

[0058] In certain preferred methods, a gossypol compound is provided to a subject in a dose that sensitizes the subject to treatment by one or more second agents. The present invention provides compositions and methods directed at therapeutic treatment of resistant diseases (e.g., cancer). Diseases that are specifically contemplated by the present invention include, but are not limited to, chemotherapy resistant diseases (e.g., cancers) and radiation therapy resistant diseases (e.g., cancers). In particularly preferred embodiments, the administration of gossypol compound(s), and optionally one or more chemotherapeutic agents (e.g., anticancer drug) or therapeutic methods (e.g., radiation therapy) sensitizes the disease (e.g., disease cells) to treatment.

[0059] In some embodiments, the hyperproliferative (or neoplastic) disease is a cancer (e.g., breast cancer, prostate cancer, pancreatic cancer, colon cancer, lung cancer, lymphoma, melanoma, or head-neck cancer). The present invention contemplates treating metastatic cancers.

[0060] The present invention further provides compositions (e.g., pharmaceutical formulations) and methods for treating diseases (e.g., cancer) the use of which in a subject results in the regression of the disease. In other embodiments, the use of the compositions and methods of the present invention in a subject having a disease (e.g., cancer) results in the arrest or stasis of a disease.

[0061] The present invention further provides a pharmaceutical composition for the treatment of tumors characterized in that it comprises a gossypol compound and an additional therapeutic agent. Similarly, also provided are pharmaceutical compositions comprising a gossypol compound and an additional therapeutic agent, wherein the pharmaceutical composition is useful as an anti-tumor therapy.

[0062] In certain pharmaceutical compositions the gossypol compound is selected from the group comprising (−)-gossypol, (+)-gossypol, (−)-gossypolone, (+)-gossypolone, (−)-gossypol acetic acid, (+)-gossypol acetic acid, (−)-ethyl gossypol, (+)-ethyl gossypol, (−)-hemigossypolone, (+)-hemigossypolone, a Schiff's base of (−)-gossypol, a Schiff's base of (+)-gossypol, a Schiff's base of (−)-gossypolone, a

Schiff's base of (+)-gossypolone, a Schiff's base of (−)-gossypol acetic acid, a Schiff's base of (+)-gossypol acetic acid, a Schiff's base of (−)-ethyl gossypol, a Schiff's base of (+)-ethyl gossypol, a Schiff's base of (−)-hemigossypolone, and a Schiff's base of (+)-hemigossypolone, (−)-apogossypol, (+)-apogossypol, (−)-apogossypol acetic acid, (+)-apogossypol acetic acid, (−)-ethyl apogossypol, (+)-ethyl apogossypol, or the racemate of any of the above enantiomeric pairs.

[0063] In still other pharmaceutical compositions and therapeutic methods the target tumor is selected from the group consisting of breast cancer, prostate cancer, lymphoma, skin cancer, pancreatic cancer, colon cancer, melanoma, malignant melanoma, ovarian cancer, brain cancer, primary brain carcinoma, head-neck cancer, glioma, glioblastoma, liver cancer, bladder cancer, non-small cell lung cancer, head or neck carcinoma, breast carcinoma, ovarian carcinoma, lung carcinoma, small-cell lung carcinoma, Wilms' tumor, cervical carcinoma, testicular carcinoma, bladder carcinoma, pancreatic carcinoma, stomach carcinoma, colon carcinoma, prostatic carcinoma, genitourinary carcinoma, thyroid carcinoma, esophageal carcinoma, myeloma, multiple myeloma, adrenal carcinoma, renal cell carcinoma, endometrial carcinoma, adrenal cortex carcinoma, malignant pancreatic insulinoma, malignant carcinoid carcinoma, choriocarcinoma, mycosis fungoides, malignant hypercalcemia, cervical hyperplasia, leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, chronic granulocytic leukemia, acute granulocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, hairy cell leukemia, neuroblastoma, rhabdomyosarcoma, Kaposi's sarcoma, polycythemia vera, essential thrombocytosis, Hodgkin's disease, non-Hodgkin's lymphoma, soft-tissue sarcoma, osteogenic sarcoma, primary macroglobulinemia, and retinoblastoma.

[0064] The present invention further provides pharmaceutical compositions, wherein an additional therapeutic agent (one or more second agents) is selected from the group consisting of agents that induce apoptosis, pro-apoptotic Bcl-2 proteins, polynucleotides, polypeptides, photodynamic compounds, radiodynamic compounds, radionuclides, radioactive elements, gamma ray emitters, beta particle emitters, drugs, biological mimetics, alkaloids, alkylating agents, antibiotics, antimicrobials, antifungals, antimetabolites, hormones, platinum compounds, monoclonal antibodies, toxins, defensins, interferons, interleukins, adoptive immunotherapy agents, hematopoietic growth factors, agents that induce tumor cell differentiation, gene therapy reagents, antisense molecules, kinase inhibitors, vascular growth factor receptor kinase inhibitor, fibroblast growth factor receptor kinase inhibitor, platelet-derived growth factor receptor kinase inhibitor, GLEEVEC, anti-estrogens, anti-androgens, cyclooxygenase 2 (COX-2) inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), chemotherapeutic drugs, nucleotide analogue reverse transcriptase inhibitors, nucleoside analogue reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, and combinations thereof. In certain embodiments, the additional therapeutic agent (or one or more second agent) is selected from the group consisting of 3,7,11,15-tetramethyl-2,6,10,14-hexadecatraen-1-ol, a Bcl-2 family protein (e.g., Bax, Bak, Bid, Bad), DNA, RNA, ribozymes, RNAse, siRNAs, enzymes, ^{111}In-oxine, ^{59}Fe, ^{67}Cu, ^{125}I, ^{99}Te, ^{51}Cr, ^{32}P, ^{3}H, ^{35}S, ^{14}C, IFN-α, IL-2, all-trans-retinoic acid, EGFR, VGFR, FGFR, PDGFR, STI-571, GLEEVEC, HERCEPTIN, RITUXAN, raloxifene, tamoxifen, flutamide, bicalutamide, finasteride, aminoglutethamide, ketoconazole, corticosteroids, celecoxib, meloxicam, NS-398, irinotecan CPT-11, fludarabine, dacarbazine, dexamethasone, mitoxantrone, MYLOTARG, VP-16, 5-FU, cisplatin, carboplatin, gemcitabine, doxorubicin, TAXOTERE, TAXOL, tenofovir disoproxil fumarate, zidovudine, lamivudine, abacavir, zalcitabine, didanosine, stavudine, nevirapine, delavirdine, efavirenz, saquinavir (SQV (HGC)), saquinavir (SQV (SGC)), ritonavir, indinavir, nelfinavir, amprenavir, mechlorethamine, cyclophosphamide, ifosfamide, melphalan, L-sarcolysin, chlorambucil, hexamethylmelamine, thiotepa, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, methotrexate, fluorouracil, floxuridine, cytarabine, mercaptopurine, thioguanine, pentostatin, vinblastine, vincristine, etoposide, teniposide, dactinomycin, daunorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, hydroxyurea, procarbazine, mitotane, aminoglutethimide, prednisone, hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, ethinyl estradiol, testosterone propionate, fluoxymesterone, flutamide, and leuprolide, and combinations thereof.

[0065] In still some other embodiments, the present invention provides compositions and methods for preventing (or attenuating) the onset or spread of a hyperproliferative disease. In some other embodiments, the present invention provides compositions and methods for preventing (or attenuating) the onset or spread of a neoplastic disease. In some preferred embodiments, the present invention provides methods of preventing (or attenuating) cancers in a subject comprising administering to the subject a gossypol compound (e.g., (−)-gossypol, (−)-gossypol acetic acid, etc.) in an amount effective to inhibit Bcl-2 family protein (e.g., Bcl-2 and/or Bcl-X_L). In some of these embodiments, the Bcl-2 family proteins contemplated include, but are not limited to, Bcl-2, Bcl-X_L, Mcl-1, Bcl-w, A1/BFL-1, BOO-DIVA, Bcl-6, Bcl-8, and Bcl-y.

[0066] Preferably, methods of preventing (or attenuating) hyperproliferative and/or neoplastic diseases comprise a gossypol compound administered in conjunction with another agent or treatment, such as an anticancer agent, an anti-neoplastic agent (e.g., a tumor cell apoptosis promoting agent), or radiation therapy. The present methods of preventing hyperproliferative and/or neoplastic diseases are not limited to the administration of any particular gossypol compound. Indeed, the present invention contemplates that a number of gossypol compounds can be administered to a subject to prevent (or attenuate) hyperproliferative and/or neoplastic diseases including, but not limited to, (±)-gossypol; (−)-gossypol; (+)-gossypol; (±)-gossypolone; (−)-gossypolone; (+)-gossypolone; (±)-gossypol acetic acid; (−)-gossypol acetic acid; (+)-gossypol acetic acid; (±)-ethyl gossypol; (−)-ethyl gossypol; (+)-ethyl gossypol; (±)-hemigossypolone; (−)-hemigossypolone; (+)-hemigossypolone; Schiff's base of (±)-gossypol; Schiff's base of (−)-gossypol; Schiff's base of (+)-gossypol; Schiff's base of (±)-gossypolone; Schiff's base of (−)-gossypolone; Schiff's base of (+)-gossypolone; Schiff's base of (±)-gossypol acetic acid; Schiff's base of (−)-gossypol acetic acid; Schiff's base of (+)-gossypol acetic acid; Schiff's base of (±)-ethyl gossypol; Schiff's base of (−)-ethyl gossypol; Schiff's base of (+)-ethyl gossypol; Schiff's base of (±)-hemigossypolone; Schiff's

base of (−)-hemigossypolone; Schiff's base of (+)-hemigossypolone, (±)-apogossypol, (−)-apogossypol, (+)-apogossypol, (±)-apogossypol acetic acid, (−)-apogossypol acetic acid, (+)-apogossypol acetic acid, (±)-ethyl apogossypol, (−)-ethyl apogossypol, (+)-ethyl apogossypol, and the like.

[0067] Similarly, the present compositions and methods of preventing (or attenuating) a hyperproliferative and/or neoplastic disease are not limited to any particular additional (second) chemotherapeutic, anticancer, or anti-neoplastic agents or therapies. The present invention contemplates that any of the exemplary therapeutics described herein (or referenced herein) may find use in certain embodiments.

[0068] Those skilled in the art can determine the amount of attenuation or whether prevention of a hyperproliferative and/or neoplastic disease has occurred upon use of the compositions and methods of the present invention in a subject, or in in vitro or ex vivo cells, tissues, and organs using standard protocols in comparison to nonpathological subjects, cells, tissues, and organs.

[0069] Still further embodiments of the present invention provide the use of a gossypol compound and an additional therapeutic agent in the manufacture of a medicament for the treatment of a neoplastic and/or hyperproliferative disease.

[0070] Other embodiments of the present invention specifically contemplate chemical intermediates, and formulations of compounds (e.g., gossypol compounds and optionally one or more chemotherapeutic agents) used in medicaments, in the manufacture of medicaments, kits for the administration of medicaments, or diagnostic tests and other applications related thereto, and other beneficial formulations.

[0071] Also provided are uses of the compositions and methods of the present invention for the preparation of therapeutics, medicaments, and other therapeutic applications.

[0072] In yet other embodiments, the present invention provides methods and compositions according to any of the claims or substantially as described in any of the Examples or various embodiments disclosed herein.

[0073] Other advantages, benefits, and preferable embodiments of the present invention will be apparent to those skilled in the art.

DESCRIPTION OF THE FIGURES

[0074] The following figures form part of the specification and are included to further demonstrate certain aspects and embodiments of the present invention. The present invention is not intended to be limited however to the embodiments specifically recited in these figures.

[0075] The following figures form part of the specification and are included to further demonstrate certain aspects and embodiments of the present invention. The present invention is not intended to be limited however to the embodiments specifically recited in these figures.

[0076] **FIG. 1** shows a sequence alignment of Bcl-2 (SEQ ID NO:1) and Bcl-X_L (SEQ ID NO:2).

[0077] **FIG. 2A** shows a ribbon representation of the overall Bcl-2 structure in complex with the Bak BH3 peptide modeled from the structure of Bcl-X_L in complex with Bak BH3 peptide. **FIG. 2B** shows a detailed representation of the BH3 binding site in Bcl-2.

[0078] **FIG. 3** shows gossypol directly inhibits binding between Bak BH3 peptide and Bcl-2, and between Bak BH3 peptide and Bcl-X_L proteins in certain fluorescence polarization (FP) based binding assays.

[0079] **FIG. 4** shows the results of competitive inhibition assays using racemic gossypol, (−)-gossypol, and (+)-gossypol to directly block binding between Bid 21-residue BH3 peptide and Bcl-2.

[0080] **FIG. 5** shows the results of a competitive inhibition assay using racemic gossypol, (−)-gossypol, and (+)-gossypol to directly block binding between Bad 25-residue BH3 peptide and Bcl-X_L.

[0081] **FIG. 6A** shows the results of a FP-based binding assay of racemic gossyplone to Bcl-X_L in one embodiment of the present invention. **FIG. 6B** shows the results of a FP-based binding assay of a ethyl Schiff's base of (−)-gossypol to Bcl-X_L (time-course) in one embodiment of the present invention.

[0082] **FIG. 7** shows the results of several cancer cell lines and one normal cell line that express various levels of Bcl-2 and/or Bcl-X_L proteins in one embodiment of the present invention.

[0083] **FIGS. 8A and 8B** show the results of cell based assays in various embodiments of the present invention.

[0084] **FIG. 9** shows the results of cell based assays in various embodiments of the present invention.

[0085] FIGS. **10** shows the results of cell based assays in various embodiments of the present invention.

[0086] **FIGS. 11A and 11B** show the results of the interactions between (−)-gossypol and Bcl-X_L protein using ^{15}N Heteronuclear Single Quantum Coherence Spectroscopy (HSQC) NMR methods in various embodiments of the present invention. **FIG. 11C** shows the three-dimensional structural representation of (−)-gossypol in complex with Bcl-X_L protein based upon NMR experimental data and computational modeling in one embodiment of the present invention. The Bcl-X_L protein is represented in a ribbon model and the (−)-gossypol is represented in a stick model.

[0087] **FIG. 12** shows the results of cell based assays in one embodiment of the present invention.

[0088] **FIG. 13** shows the results of cell based assays in one embodiment of the present invention.

[0089] **FIG. 14** shows the results of cell based assays in one embodiment of the present invention.

[0090] **FIG. 15** shows the results of in vivo animal xenograft based assays in one embodiment of the present invention.

[0091] **FIG. 16** shows the results of cell based assays in various embodiments of the present invention.

[0092] **FIGS. 17A and 17B** show the results of cell based assays in various embodiments of the present invention.

[0093] **FIG. 18** shows the results of in vivo animal xenograft based assays in various embodiments of the present invention.

[0094] **FIG. 19** shows the results of in vivo animal xenograft based assays in various embodiments of the present invention.

[0095] **FIG. 20** shows the results of in vivo animal xenograft based assays in one embodiment of the present invention.

[0096] **FIG. 21** shows the results of cell based assays (inhibition of cell growth in several head-neck cancer cell lines and three fibroblast cell lines) by (−)-gossypol in one embodiment of the present invention.

[0097] **FIG. 22** show the results of Western blotting analysis of the protein levels of Bcl-2, Bcl-X_L and Bcl-X_S in several head-neck cancer cell lines and one fibroblast cell line in various embodiments of the present invention.

[0098] **FIG. 23** show the results of cell growth inhibition by (−)-gossypol in a panel of head-neck cancer cell lines and one fibroblast cell line as determined by an MTT assay (right Y-axis) and its relationship with the ratio of Bcl-X_L/Bcl-X_S (left Y-axis) in various embodiments of the present invention.

[0099] **FIGS. 24A-24C** show the results of apoptosis induction studies using (−)-gossypol in 6 cell lines as determined by the TUNEL assay (UM-SCC-1, UM-SCC-6, UM-SCC-12, UM-SCC-14A, fibroblast 1 and fibroblast 2) in various embodiments of the present invention.

[0100] **FIG. 25** shows the chemical structures of gossypol, gossyplone, Schiff's bases of gossypol and Schiff's bases of gossypolone, (−)-gossypol and (+)-gossypol in various embodiments of the present invention.

[0101] **FIG. 26** shows the results of a saturation curve of Bcl-X_L protein to Bad 25-residue BH3 peptide.

[0102] **FIG. 27** shows the results of saturation curve of Bcl-2 protein binding to Bid 21-residue BH3 peptide.

[0103] **FIGS. 28A and 28B** show the results of nuclear magnetic resonance (NMR) based binding assays of (−)-gossypol and (+)-gossypol to Bcl-X_L, respectively.

[0104] FIGS. **29** shows the results of cell based assays in various embodiments of the present invention.

[0105] FIGS. **30** shows the results of cell based assays of gossypol, (−)-gossypol, and (+)-gossypol in various embodiments of the present invention.

[0106] **FIG. 31** shows the results of cell based assays in one embodiment of the present invention.

[0107] **FIG. 32A and 32B** shows the results of cell based assays in one embodiment of the present invention.

[0108] **FIG. 33** shows the results of cell based colony formation assays in one embodiment of the present invention.

[0109] **FIG. 34** shows the results of cell based assays in various embodiments of the present invention.

[0110] **FIGS. 35A and 35B** show the results of cell based assays in one embodiment of the present invention.

[0111] **FIG. 36** shows the results of in vivo animal xenograft based assays in one embodiment of the present invention.

[0112] **FIG. 37** shows the results of in vivo animal xenograft based assays in one embodiment of the present invention.

[0113] **FIG. 38** shows the results of in vivo animal xenograft based assays in one embodiment of the present invention.

[0114] FIGS. **39** shows the results of cell based assays of the inhibition of cell growth in 2 prostate cancer cell lines PC-3 and LnCaP by racemic gossypol, (−)-gossypol, and (+)-gossypol in various embodiments of the present invention.

[0115] **FIG. 40** shows the results of cell based assays in one embodiment of the present invention.

[0116] **FIG. 41** shows the results of Western blotting analysis of several Bcl-2 family proteins in one embodiment of the present invention.

[0117] **FIG. 42** shows the results of cell based assays in one embodiment of the present invention.

[0118] **FIGS. 43A and 43B** show the results of in vivo animal xenograft based assays in various embodiments of the present invention.

[0119] **FIG. 44** shows the results of cell based assays in one embodiment of the present invention.

[0120] **FIG. 45** describes the competitive binding curve of racemic apogossypol in directly blocking binding between Bad 25-residue BH3 peptide and Bcl-2 using an in vitro fluorescence polarization-based assay.

[0121] **FIG. 46** describes the competitive binding curve of racemic apogossypol in directly blocking the binding between Bad 21-residue BH3 peptide and Bcl-X_L protein using an in vitro fluorescence polarization-based assay.

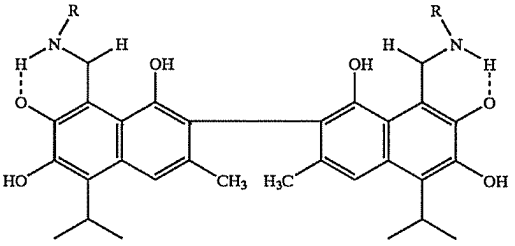
DEFINITIONS

[0122] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

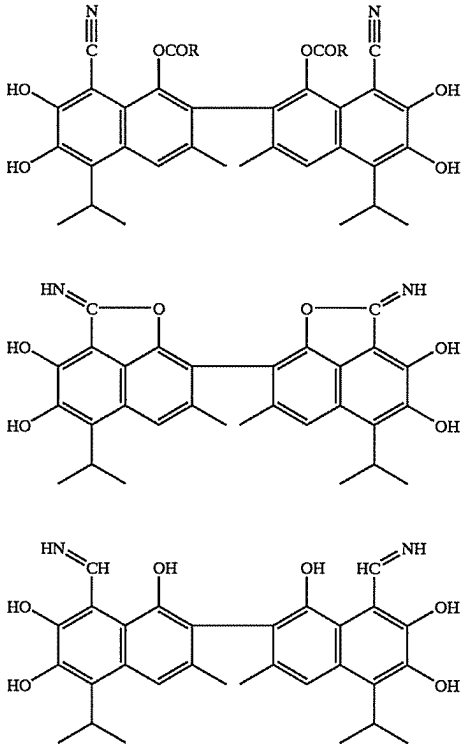
[0123] As used herein, the term "gossypol compound" refers to enantiomers, isomers, derivatives, metabolites, Schiff's bases, combinations with acids or bases, and pharmaceutically acceptable salts of the gossypol molecule. Accordingly, gossypol compounds include, but are not limited to, (±)-gossypol; (−)-gossypol; (+)-gossypol; (±)-gossypolone; (−)-gossypolone; (+)-gossypolone; (±)-gossypol acetic acid; (−)-gossypol acetic acid; (+)-gossypol acetic acid; (±)-ethyl gossypol; (−)-ethyl gossypol; (+)-ethyl gossypol; (±)-hemigossypolone; (−)-hemigossypolone; (+)-hemigossypolone; Schiff's base of (±)-gossypol; Schiff's base of (−)-gossypol; Schiff's base of (+)-gossypol; Schiff's base of (±)-gossypolone; Schiff's base of (−)-gossypolone; Schiff's base of (+)-gossypolone; Schiff's base of (±)-gossypol acetic acid; Schiff's base of (−)-gossypol acetic acid; Schiff's base of (+)-gossypol acetic acid; Schiff's base of (±)-ethyl gossypol; Schiff's base of (−)-ethyl gossypol; Schiff's base of (+)-ethyl gossypol; Schiff's base of (±)-hemigossypolone; Schiff's base of (−)-hemigossypolone; Schiff's base of (+)-hemigossypolone, (±)-apogossypol, (−)-apogossypol, (+)-apogossypol, (±)-apogossypol acetic acid, (−)-apogossypol acetic acid, (+)-apogossypol acetic acid, (±)-ethyl apogossypol, (−)-ethyl apogossypol, (+)-ethyl apogossypol. Acids that may be used in combination with

gossypol include, but are not limited to, formic acid, acetic acid, propionic acid, and butyric acid. Physiologically acceptable salts include, but are not limited to, salts comprising sodium hydroxide, potassium hydroxide, lithium hydroxide, barium hydroxide, sodium carbonate, potassium carbonate, sodium acetate, potassium acetate, pyridine, triethylamine, and quinoline.

[0124] Gossypol derivatives include any derivatives that are useful in the present invention. One of skill in the art is familiar with derivatization techniques. Many gossypol derivatives are known including, but not limited to, the following compounds:



[0125] wherein R=methyl, ethyl, propyl, isopropyl, butyl, s-butyl, t-butyl, pentyl, hexyl, heptyl, dodecyl, β-methyl phenylalanine ethyl, phenylalanine methyl ester (Razakantoanina et al. Parasitol. Res., 86:665-668 (2000));



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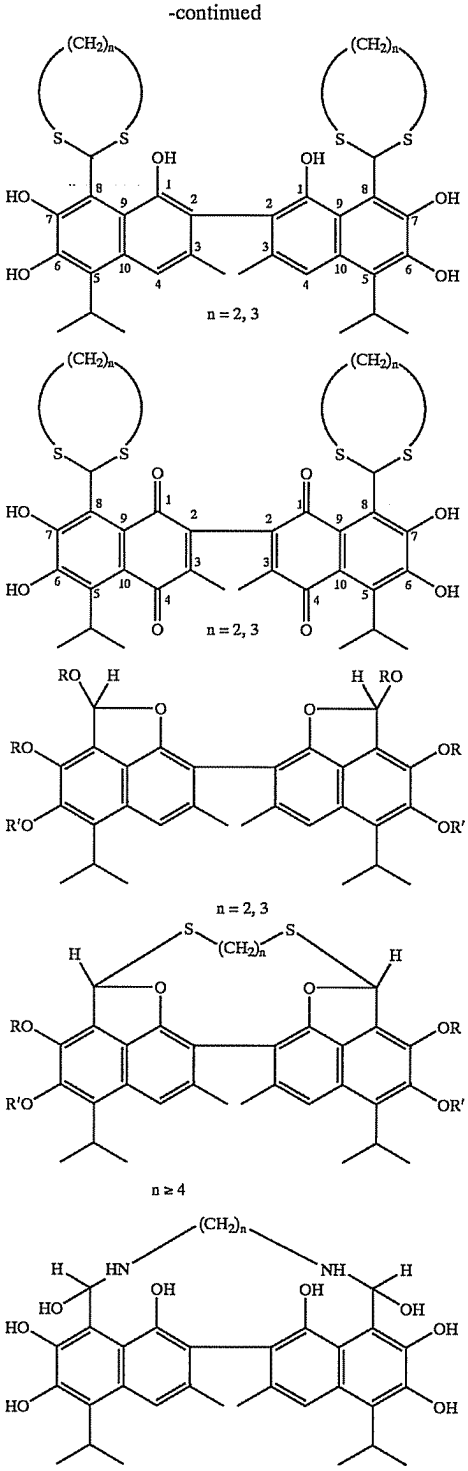

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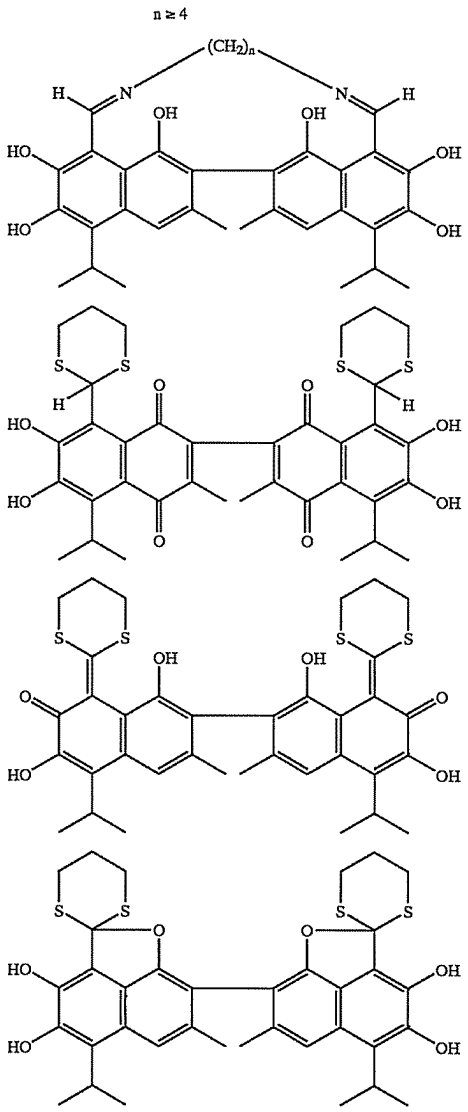
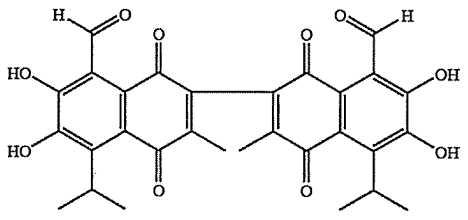


[0126] wherein R=methyl and R'=hydrogen, methyl (Dao et al. Bioorg. Med. Chem., 11:2001-2006 (2003));



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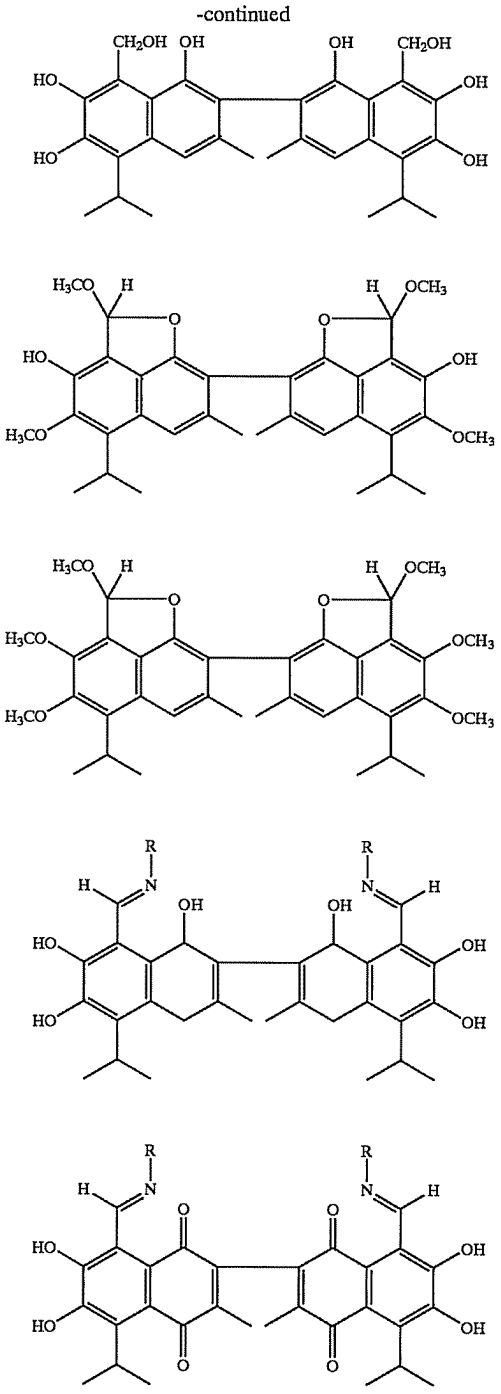
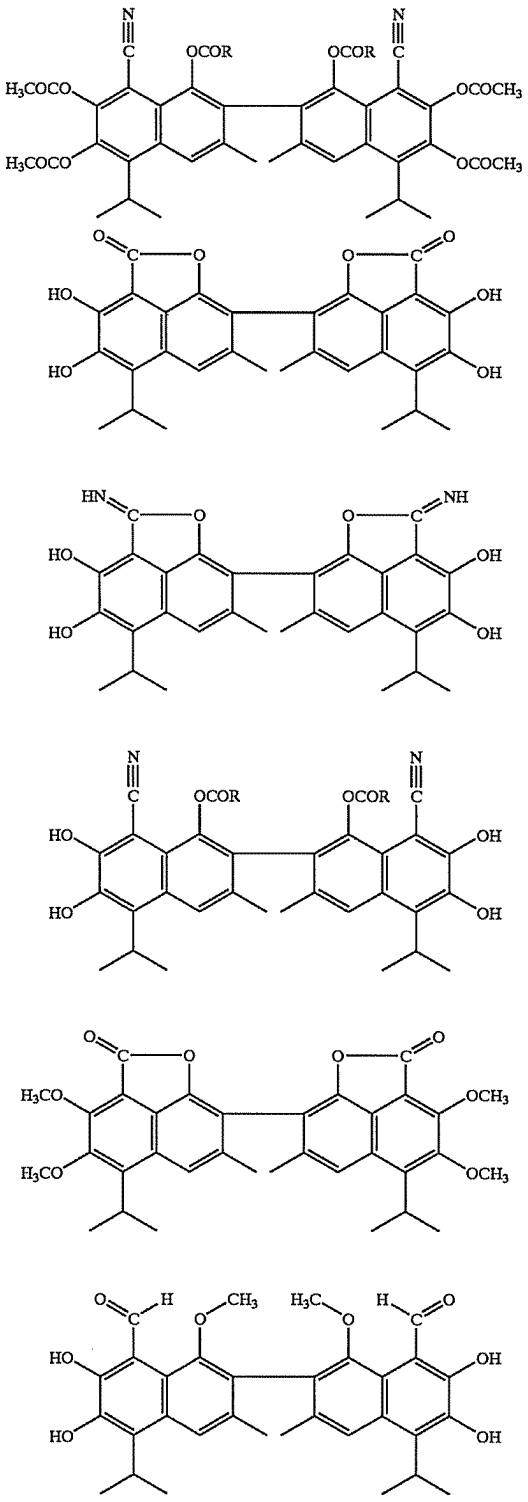


[0127] wherein R=methyl, ethyl, propyl, isopropyl, butyl, s-butyl, t-butyl, pentyl, hexyl, heptyl, dodecyl, β-methyl phenylalanine ethyl, phenylalanine methyl ester (Dao et al. Eur. J. Med. Chem., 35:805-813 (2000));



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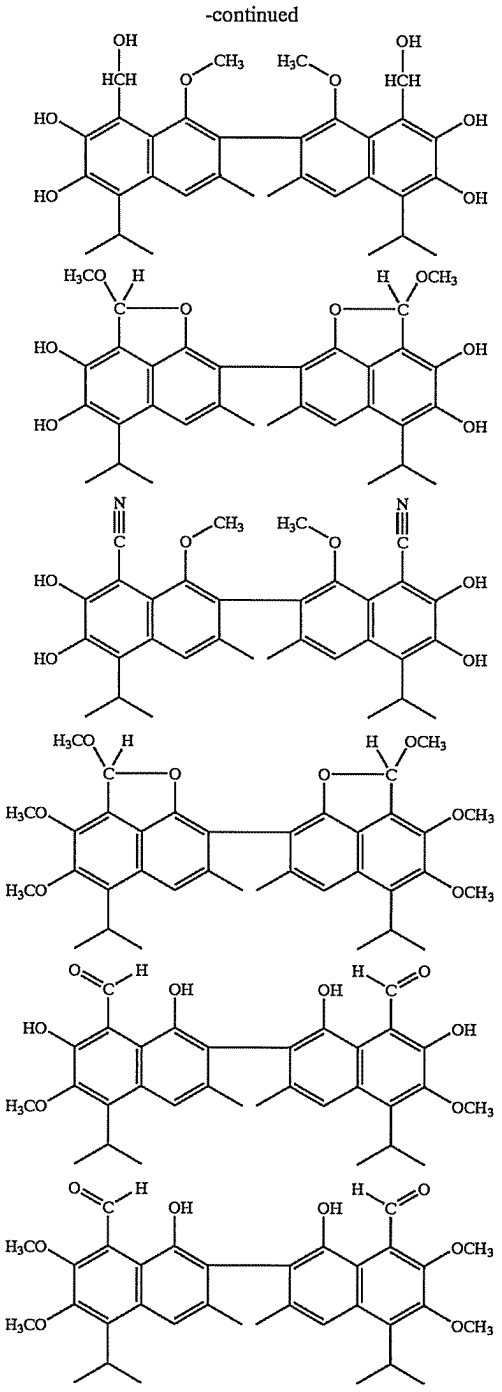
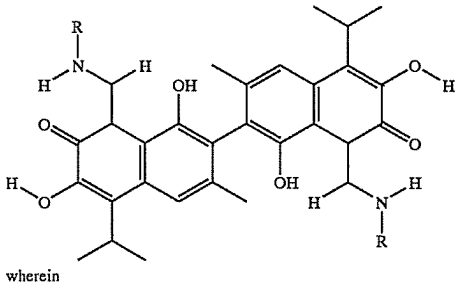
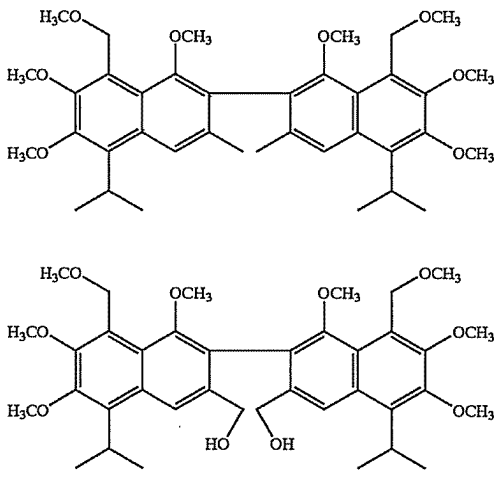


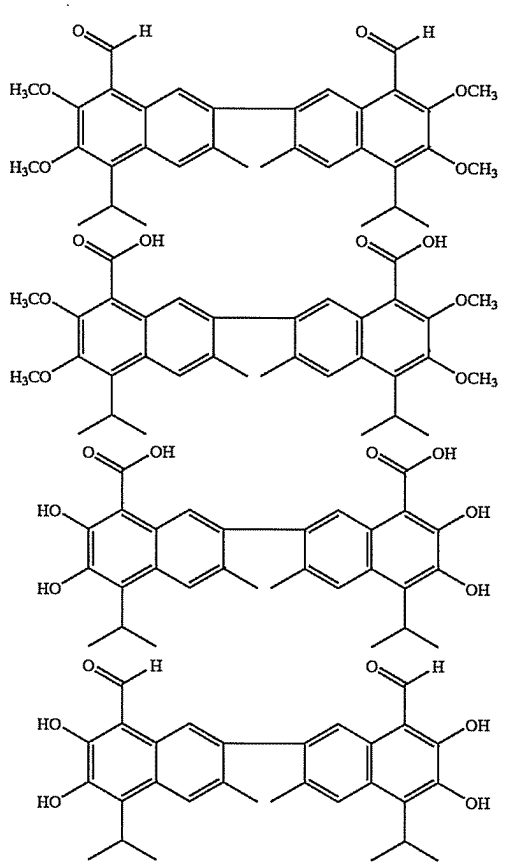
[0128] wherein R=methyl, ethyl, propyl, butyl, pentyl, propenyl, or t-butyl (Deck et al. J. Med. Chem., 34:3301-3305 (1991));



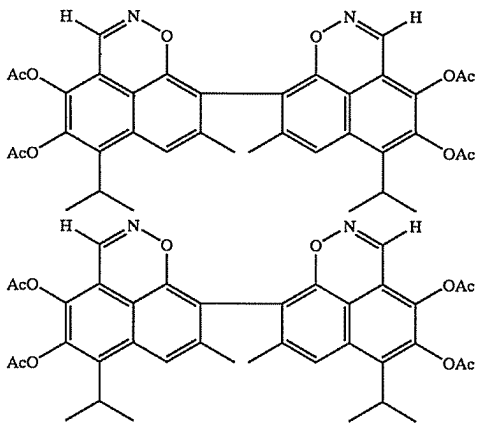
[0129] Przybylski etal. J. Mol. Structure, 611(1-3):193-201 (2002);



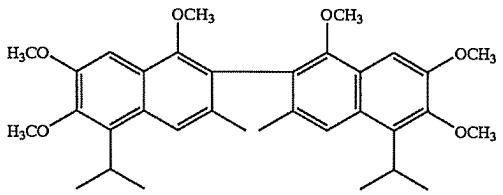
[0130] (A. I. Meyers and J. Jeffrey Willemsen, Chem. Commun., 16:1573-1574 (1997));

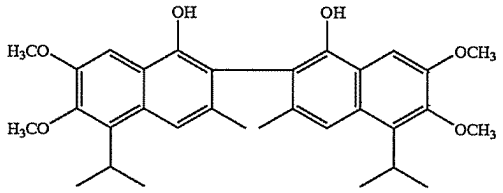


[0131] (R. E. Royer et al., J. Med. Chem., 38:2427-2432 (1995));

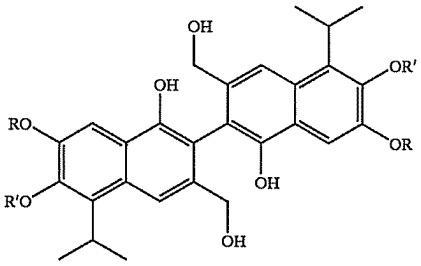


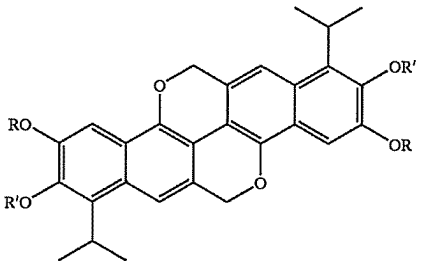
[0132] (R. E. Royer et al., J. Med. Chem., 29:1799-1801 (1986));

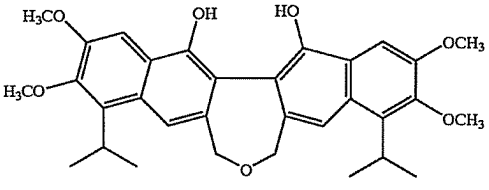




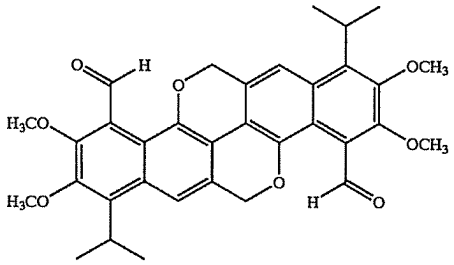
[0133] (C. M. Venuti, J. Org. Chem., 46(15):3124-3127 (1981));

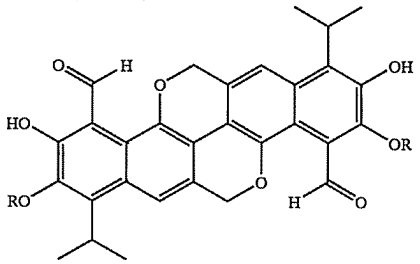


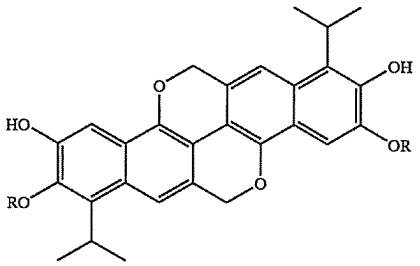




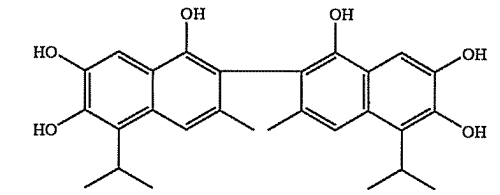
[0134] wherein R=Me, Bz and R'=Me, H, and Bz;

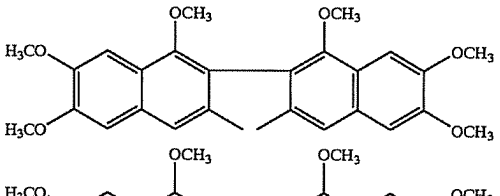


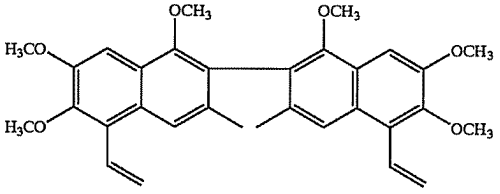




[0135] wherein R=Me, and H, (I. V. Ognyanoc et al., Helv. Chim. Acta, 72:353-360 (1989));







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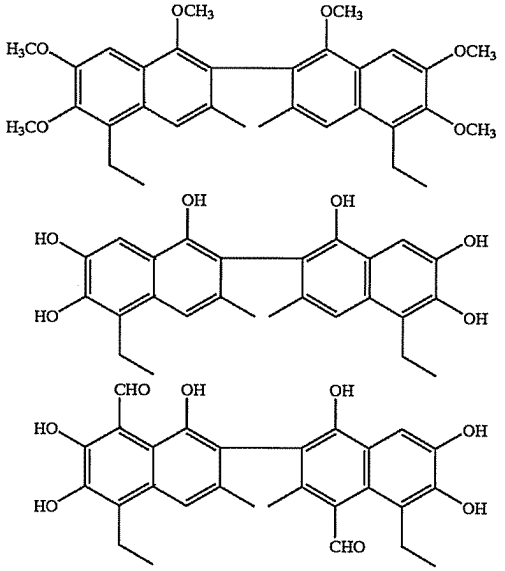
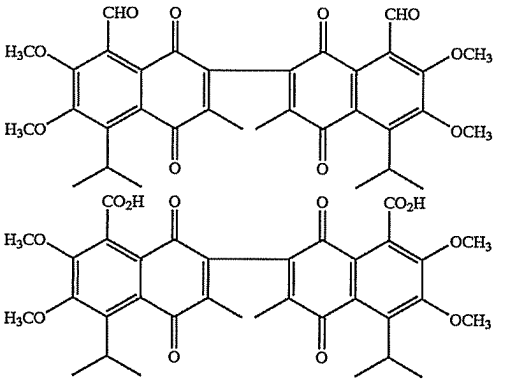


[0136] (P.C. Meltzer et al., J. Org. Chem., 50(17):3121-3124 (1985));



[0137] (R. Adams et al., J. Am. Chem. Soc., 60:2193-2204 (1938)). Other derivatives of gossypol are disclosed in the following references: Le Blanc et al. Pharmacol. Res., 46:551-555 (2002); Baumgrass et al. J. Biol. Chem., 276:47914-47921 (2001); Shelley et al. Anticancer Drugs, 11:209-216 (2000); Sonenberg et al. Contraception, 37:247-255, (1988); Whaley et al. Contraception, 33:605-616 (1986); Dorsett et al. J. Pharm. Sci., 64:1073-1075 (1975); Wu et al. Yao Xue Xue Bao, 24:502-511 (1989); Hoffer et al. Contraception, 37:301-331 (1988); Guo et al. Yao Xue Xue Bao, 22:597-602 (1987); and Manmade et al. Experientia, 39:1276-1277 (1983).

[0138] As used herein, the term "gossypol acetic acid" refers to a composition of gossypol comprising an amount of acetic acid sufficient to detectably stabilize the gossypol composition as compared to gossypol compositions without acetic acid. The range of acetic acid in "gossypol acetic acid" compositions is preferably from about 0.01% to 99% (by weight), more preferably from about 0.1% to 50%, even more preferably from about 0.5% to 20%. In one embodiment, the gossypol acetic acid is a complex consisting of equimolar quantities of gossypol and acetic acid (Sigma-Aldrich Corp., St. Louis, Mo.).

[0139] As used herein, the terms "(−)-gossypol," or "(−)-gossypol compound/composition," refer to an optically active composition of gossypol wherein the active molecules comprising the composition rotate plane polarized light counterclockwise (e.g., levorotatory molecules) as measured by a polarimeter. Preferably, the (−)-gossypol compound has an enantiomeric excess of 1% to 100%. In one embodiment, the (−)-gossypol compound has an enantiomeric excess of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (−)-gossypol. In one example of a "(−)-gossypol compound", the specific rotation ($[\alpha]_D$) of the compound is about −350° to about −390°, about −375° to about −390°, or about −385° to about −390°. (See e.g., Dowd, Chirality, 15:486 (2003); Ciesielska et al., Chem. Phys. Lett. 353:69 (2992); Freedman et al., Chirality, 15:196 (2003); and Zhou et al., Kexue Tongbao, 28:1574 (1983)). Methods for resolving racemic gossypol compounds into substantially purified (+)- or (−)-gossypol are known (See e.g., Zhou et al., Kexue Tongbao, 28:1574 (1983) (wherein: L-phenylalanine methyl ester was mixed with the aldehyde groups of gossypol to form a Schiff's base with two diastereoisomers which were then resolved on a normal silica flash chromatography column. The filtrate was concentrated, and the residue was purified by chromatography on silica gel eluting with hexanes:EtOAc=3:1 to give two fractions. Acid hydrolysis of the two fractions in 5N HCl:THF (1:5, room temperature, overnight) regenerated the individual gossypol enantiomers, respectively. The first fraction with a higher R_f value contained (−)-gossypol, and the second fraction with a lower R_f value contained (+)-gossypol. The crude gossypol fractions were extracted into ether from the residue after removing THF from the reaction mixture. The gossypol fractions were then purified by chromatography on silica gel and eluted with hexanes:EtOAc (3:1 ratio) to give optically pure gossypol, with a yield of 30-40% in two steps. The optical rotatory dispersion values for these products were α_D=−352° (c=0.65, $CHCl_3$) for (−)-gossypol, and α_D=+341° (c=0.53, $CHCl_3$)).

[0140] As used herein, the term "gossypol Schiff's base(s)" refers to the gossypol compound that results from the reaction of an aldehyde or ketone form of gossypol with a primary amine to yield an imine of gossypol. Examples of primary amines that can be used include, but are not limited to, branched and unbranched alkylamines (e.g., methylamine, ethylamine, propylamine, isopropylamine, butylamine, t-butylamine), substituted and unsubstituted arylamines (e.g., phenylamine, benzylamine), and amino acids, such as glycine, alanine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, lysine, arginine, histidine, aspartate, glutamate, asparagine, glutamine, cysteine, and methionine.

[0141] As used herein, the term "Bcl-2 family proteins," refers to both the anti-apoptotic members of the Bcl-2 family, including, but not limited to Bcl-2, Bcl-X_L, Mcl-1, A1/BFL-1, BOO-DIVA, Bcl-w, Bcl-6, Bcl-8 and Bcl-y, and

the pro-apoptotic members of the Bcl-2 family, including, but not limited to Bak, Bax, Bad, tBid, Harakiri, Bim, Bmf, and optionally other proteins with BH3 (Bcl-2 homology 3) binding pockets that are regulated by gossypol compounds.

[0142] As used herein, the terms "overexpression of Bcl-2," or "overexpression of a Bcl-2 family protein" refer to an elevated level (e.g., aberrant) of mRNAs encoding for a Bcl-2 family protein(s), and/or to elevated levels of such Bcl-2 family protein(s) in cells or tissues as compared to similar normal corresponding nonpathological cells and tissues expressing basal levels of mRNAs encoding Bcl-2 family proteins or having basal levels of Bcl-2 family proteins. Methods for detecting the levels of mRNAs encoding Bcl-2 family proteins, or levels of Bcl-2 family proteins, in a cell or tissue include, but are not limited to, Western blotting using Bcl-2 family protein antibodies, immunohistochemical methods, and methods of nucleic acid amplification or direct RNA detection. As important as the absolute levels of Bcl-2 family proteins in cells, tissues, or organs are to determining that they overexpress Bcl-2 family proteins, so also are the relative levels of anti-apoptotic Bcl-2 family proteins to other pro-apoptotic signalling molecules (e.g., pro-apoptotic Bcl-2 family proteins) within such cells, tissues or organs. When the balance of these two are such that, were it not for the levels of the anti-apoptotic Bcl-2 family proteins, the pro-apototic signalling molecules would be sufficient to cause the cells to execute the apoptosis program and die, said cells in such tissues or organs would be dependent on the anti-apoptotic Bcl-2 family proteins for their survival. In such cells, exposure to an inhibiting effective amount of an anti-apoptotic Bcl-2 family protein inhibitor will be sufficient to cause the cells to execute the apoptosis program and die. Thus, the term "overexpression of Bcl-2 family protein" also refers to cells in tissues and organs that, due to the relative levels of pro-apoptotic signals and anti-apoptotic signals, undergo apoptosis in response to inhibiting effective amounts of compounds that inhibit the finction of anti-apoptotic Bcl-2 proteins.

[0143] As used herein, the terms "anticancer agent,""conventional anticancer agent," or "cancer therapeutic drug" refer to any therapeutic agents (e.g., chemotherapeutic coumpounds and/or molecular therapeutic compounds), radiation therapies, or surgical interventions, used in the treatment of cancer (e.g., in mammals).

[0144] As used herein, the terms "drug" and "chemotherapeutic agent" refer to pharmacologically active molecules that are used to diagnose, treat, or prevent diseases or pathological conditions in a physiological system (e.g., a subject, or in vivo, in vitro, or ex vivo cells, tissues, and organs). Drugs act by altering the physiology of a living organism, tissue, cell, or in vitro system to which the drug has been administered. It is intended that the terms "drug" and "chemotherapeutic agent" encompass anti-hyperproliferative and antineoplastic compounds as well as other biologically therapeutic compounds.

[0145] As used herein the term "prodrug" refers to a pharmacologically inactive derivative of a parent "drug" molecule that requires biotransformation (e.g., either spontaneous or enzymatic) within the target physiological system to release, or to convert (e.g., enzymatically, mechanically, electromagnetically, etc.) the "prodrug" into the active "drug."Prodrugs" are designed to overcome problems associated with stability, toxicity, lack of specificity, or limited bioavailability. Exemplary "prodrugs" comprise an active "drug" molecule itself and a chemical masking group (e.g., a group that reversibly suppresses the activity of the "drug"). Some preferred "prodrugs" are variations or derivatives of compounds that have groups cleavable under metabolic conditions. Exemplary "prodrugs" become pharmaceutically active in vivo or in vitro when they undergo solvolysis under physiological conditions or undergo enzymatic degradation or other biochemical transformation (e.g., phosphorylation, hydrogenation, dehydrogenation, glycosylation, etc.). Prodrugs often offer advantages of solubility, tissue compatibility, or delayed release in the mammalian organism. (See e.g., Bundgard, Design of Prodrugs, pp. 7-9, 21-24, Elsevier, Amsterdam (1985); and Silverman, The Organic Chemistry of Drug Design and Drug Action, pp. 352-401, Academic Press, San Diego, Calif. (1992)). Common "prodrugs" include acid derivatives such as esters prepared by reaction of parent acids with a suitable alcohol (e.g., a lower alkanol), amides prepared by reaction of the parent acid compound with an amine (e.g., as described above), or basic groups reacted to form an acylated base derivative (e.g., a lower alkylamide).

[0146] The term "derivative" of a compound, as used herein, refers to a chemically modified compound wherein the chemical modification takes place either at a functional group of the compound, aromatic ring, or carbon backbone. Such derivatives include esters of alcohol-containing compounds, esters of carboxy-containing compounds, amides of amine-containing compounds, amides of carboxy-containing compounds, imines of amino-containing compounds, acetals of aldehyde-containing compounds, ketals of carbonyl-containing compounds, and the like.

[0147] As used herein, the term "pharmaceutically acceptable salt" refers to any salt (e.g., obtained by reaction with an acid or a base) of a compound of the present invention that is physiologically tolerated in the target subject (e.g., a mammalian subject, and/or in vivo or ex vivo, cells, tissues, or organs). "Salts" of the compounds of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, sulfonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

[0148] Examples of bases include, but are not limited to, alkali metal (e.g., sodium) hydroxides, alkaline earth metal (e.g., magnesium) hydroxides, ammonia, and compounds of formula NW_4^+, wherein W is C_{1-4} alkyl, and the like.

[0149] Examples of salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, chloride, bromide, iodide, 2-hydroxyethanesulfonate, lactate,

maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+, NH_4^+, and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like. For therapeutic use, salts of the compounds of the present invention are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

[0150] An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations.

[0151] As used herein, the term "administration" refers to the act of giving a drug, prodrug, or other agent (e.g., a gossypol compound), or therapeutic treatment (e.g., radiation therapy) to a physiological system (e.g., a subject or in vivo, in vitro, or ex vivo cells, tissues, and organs). Exemplary routes of administration to the human body can be through the eyes (opthalmic), mouth (oral), skin (transdermal), nose (nasal), lungs (inhalant), oral mucosa (buccal), ear, by injection (e.g., intravenously, subcutaneously, intratumorally, intraperitoneally, etc.) and the like.

[0152] "Coadministration" refers to administration of more than one chemical agent (e.g., a gossypol compound and/or drugs, prodrugs, etc.) or therapeutic treatment (e.g., radiation therapy) to a physiological system (e.g., a subject or in vivo, in vitro, or ex vivo cells, tissues, and organs). "Coadministration" of the respective chemical agents (e.g., a gossypol compound and/or drugs, prodrugs, etc.) and therapeutic treatments (e.g., radiation therapy) may be concurrent, or in any temporal order or physical combination.

[0153] As used herein, the term "synergistic" refers to an effect obtained when gossypol and a second agent are administered together (e.g., at the same time or one after the other) that is greater than the additive effect of gossypol and the second agent when administered individually. The synergistic effect allows for lower doses of gossypol and/or the second agent to be administered or provides greater efficacy at the same doses. The synergistic effect obtained can be at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 125%, at least 150%, at least 175%, at least 200%, at least 250%, at least 300%, at least 350%, at least 400%, or at least 500% more than the additive effect of the gossypol compound and the second agent when administered individually. For example, with respect to the treatment of cancer, the synergistic effect can be a decrease in the rate of tumor growth, a decrease in tumor mass, a decrease in the number of metastases, an increase in time to tumor progression, or an increase in survival time. As described herein, gossypol compounds (e.g., (−)-gossypol) and chemotherapeutic agents, when administered individually, often only inhibit tumor cell proliferation rather than cause regression of the tumor mass. According to the present invention, it is possible to cause actual regression of tumor mass by the administration of gossypol compounds (e.g., (−)-gossypol) and chemotherapeutic agents. The co-administration of a gossypol compound and an anticancer agent may allow for the use of lower doses of the gossypol compound and/or the anticancer agent such that the cancer is effectively treated while avoiding any substantial toxicity to the subject.

[0154] The term "sensitize," and grammatical equivalents thereof, refers to making, through the administration of a first agent(s) (e.g., a gossypol compound and optionally a chemotherapeutic agent and/or radiation), a subject, cell, tissue, or organ more susceptible, or more responsive, to the biological effects (e.g., promotion or retardation of an aspect of cellular finction including, but not limited to, cell growth, proliferation, invasion, angiogenesis, apoptosis) of a second or more agent. The "sensitizing effect" of a first agent (e.g., a gossypol compound and optionally a chemotherapeutic agent and/or radiation) on a target cell, tissue, or organ can be measured as the difference in the intended biological effect (e.g., promotion or retardation of an aspect of cellular function including, but not limited to, cell growth, proliferation, invasion, angiogenesis, apoptosis) observed upon the administration of a second or more agent with and without administration of the first agent. In this regard, the second or more agent can be exogenous to the subject, cell, tissue or organ. Further in this regard, the second or more agent can be endogenous to the subject, cell, tissue, or organ.

[0155] As used herein, the term "pharmacological properties" refers to any desirable or favorable biological activities or physicochemical characteristics of an agent (e.g., a gossypol compound) administered to a physiological system.

[0156] As used herein, the term "pharmacokinetic properties" refers to the action of an agent (e.g., a gossypol compound) in a subject, cell, tissue, or organ over a period of time including, but not limited to, the processes of absorption, distribution, localization in tissues, biotransformation, and excretion.

[0157] As used herein, the term "bioavailability" refers to any measure of the ability of a an agent (e.g., a gossypol compound) to be absorbed into a biological target fluid (e.g., blood, cytoplasm, CNS fluid, and the like), tissue, organelle or intercellular space after administration to a physiological system (e.g., a subject or in vivo, in vitro, or ex vivo cells, tissues, and organs).

[0158] As used herein, the term "biodistribution" refers to the location of an agent (e.g., a gossypol compound) in organelles, cells (e.g., in vivo or in vitro), tissues, organs, or organisms, after administration to a physiological system.

[0159] As used herein, the term "dysregulation of the process of cell death" refers to any aberration in the ability of (e.g., predisposition) a cell to undergo cell death via either necrosis or apoptosis. Dysregulation of cell death is associated with or induced by a variety of conditions, including for example, autoimmune disorders (e.g., systemic lupus erythematosus, rheumatoid arthritis, graft-versus-host disease, myasthenia gravis, Sjögren's syndrome, etc.), chronic inflammatory conditions (e.g., psoriasis, asthma and Crohn's disease), hyperproliferative disorders (e.g., tumors, B cell lymphomas, T cell lymphomas, etc.), viral infections (e.g., herpes, papilloma, HIV), and other conditions such as osteoarthritis and atherosclerosis. It should be noted that when the dysregulation is induced by or associated with a viral infection, the viral infection may or may not be

detectable at the time dysregulation occurs or is observed. That is, viral-induced dysregulation can occur even after the disappearance of symptoms of viral infection.

[0160] A "hyperproliferative disease," as used herein refers to any condition in which a localized population of proliferating cells in an animal is not governed by the usual limitations of normal growth. Examples of hyperproliferative disorders include tumors, neoplasms, lymphomas and the like. A neoplasm is said to be benign if it does not undergo invasion or metastasis and malignant if it does either of these. A "metastatic" cell or tissue means that the cell can invade and destroy neighboring body structures. Hyperplasia is a form of cell proliferation involving an increase in cell number in a tissue or organ without significant alteration in structure or function. Metaplasia is a form of controlled cell growth in which one type of fully differentiated cell substitutes for another type of differentiated cell. Metaplasia can occur in epithelial or connective tissue cells. A typical metaplasia involves a somewhat disorderly metaplastic epithelium.

[0161] The pathological growth of activated lymphoid cells often results in an autoimmune disorder or a chronic inflammatory condition. As used herein, the term "autoimmune disorder" refers to any condition in which an organism produces antibodies or immune cells which recognize the organism's own molecules, cells or tissues. Non-limiting examples of autoimmune disorders include autoimmune hemolytic anemia, autoimmune hepatitis, Berger's disease or IgA nephropathy, Celiac Sprue, chronic fatigue syndrome, Crohn's disease, dermatomyositis, fibromyalgia, graft versus host disease, Grave's disease, Hashimoto's thyroiditis, idiopathic thrombocytopenia purpura, lichen planus, multiple sclerosis, myasthenia gravis, psoriasis, rheumatic fever, rheumatic arthritis, scleroderma, Sjögren's syndrome, systemic lupus erythematosus, type 1 diabetes, ulcerative colitis, vitiligo, and the like.

[0162] As used herein, the term "neoplastic disease" refers to any abnormal growth of cells or tissues being either benign (non-cancerous) or malignant (cancerous).

[0163] As used herein, the term "anti-neoplastic agent" refers to any compound that retards the proliferation, growth, or spread of a targeted (e.g., malignant) neoplasm.

[0164] As used herein, the term "regression" refers to the return of a diseased subject, cell, tissue, or organ to a non-pathological, or less pathological state as compared to basal nonpathogenic exemplary subject, cell, tissue, or organ. For example, regression of a tumor includes a reduction of tumor mass as well as complete disappearance of a tumor or tumors.

[0165] As used herein, the terms "prevent,""preventing," and "prevention" refer to a decrease in the occurrence of hyperproliferative or neoplastic cells in a subject. The prevention may be complete, e.g., the total absence of hyperproliferative or neoplastic cells in a subject. The prevention may also be partial, such that the occurrence of hyperproliferative or neoplastic cells in a subject is less than that which would have occurred without the present invention.

[0166] As used herein the term, "in vitro" refers to an artificial environment and to processes or reactions that occur within an artificial environment. In vitro environments can consist of, but are not limited to, test tubes and cell cultures. The term "in vivo" refers to the natural environment (e.g., an animal or a cell) and to processes or reactions that occur within a natural environment.

[0167] As used herein, the term "host cell" refers to any eukaryotic or prokaryotic cell (e.g., mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located in vitro or in vivo.

[0168] As used herein, the term "cell culture" refers to any in vitro culture of cells. Included within this term are continuous cell lines (e.g., with an immortal phenotype), primary cell cultures, finite cell lines (e.g., non-transformed cells), and any other cell population maintained in vitro, including oocytes and embryos.

[0169] As used herein, the term "subject" refers to organisms to be treated by the methods of the present invention. Such organisms include, but are not limited to, humans and veterinary animals (dogs, cats, horses, pigs, cattle, sheep, goats, and the like). In the context of the invention, the term "subject" generally refers to an individual who will receive or who has received treatment (e.g., administration of gossypol compound(s), and optionally one or more anticancer agents) for a disease characterized by overexpression of Bcl-2 family proteins (e.g., Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, A-1(Bfl-1), and Boo).

[0170] The term "diagnosed," as used herein, refers to the recognition of a disease by its signs and symptoms (e.g., resistance to conventional cancer therapies), or genetic analysis pathological analysis, histological analysis, and the like.

[0171] As used herein, the term "competes for binding" is used in reference to a first molecule (e.g., a gossypol compound) with an activity that binds to the same target (e.g., Bcl-2 and/or Bcl-X_L) as does a second molecule (e.g., a pro-apoptotic Bcl-2 family protein, such as Bax, Bak, Bid, and Bad, etc.). The efficiency (e.g., kinetics or thermodynamics) of binding by the first molecule may be the same as, or greater than, or less than, the efficiency of the target binding by the second molecule. For example, the equilibrium binding constant (Kd) for binding to the target may be different for the two molecules.

[0172] As used herein, the term "antisense" is used in reference to nucleic acid sequences (e.g., RNA, phosphorothioate DNA) that are complementary to a specific RNA sequence (e.g., mRNA). Included within this definition are antisense RNA ("asRNA") molecules involved in gene regulation by bacteria. Antisense RNA may be produced by any method, including synthesis by splicing the gene(s) of interest in a reverse orientation to a viral promoter that permits the synthesis of a coding strand. For example, once introduced into an embryo, this transcribed strand combines with natural mRNA produced by the embryo to form duplexes. These duplexes then block either the further transcription of the mRNA or its translation. In this manner, mutant phenotypes may be generated. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. The designation (-) (i.e., "negative") is sometimes used in reference to the antisense strand, with the designation (+) sometimes used in reference to the sense (i.e., "positive") strand. Regions of nucleic acid sequences that are accessible to antisense molecules can be determined using available computer analysis methods.

[0173] The term "sample" as used herein is used in its broadest sense. A sample suspected of indicating a condition characterized by the overexpression of a Bcl-2 family protein may comprise a cell, tissue, or fluids, chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern blot analysis), RNA (in solution or bound to a solid support such as for Northern blot analysis), cDNA (in solution or bound to a solid support) and the like. A sample suspected of containing a protein may comprise a cell, a portion of a tissue, an extract containing one or more proteins and the like.

[0174] The term "test compound" refers to any chemical entity, pharmaceutical, drug, and the like, that can be used to treat or prevent a disease, illness, sickness, or disorder of bodily function, or otherwise alter the physiological or cellular status of a sample (e.g., the level of Bcl-2 family proteins in a cell). Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by using the screening methods of the present invention. A "known therapeutic compound" refers to a therapeutic compound that has been shown (e.g., through animal trials or prior experience with administration to humans) to be effective in such treatment or prevention. In preferred embodiments, "test compounds" are anticancer agents. In particularly preferred embodiments, "test compounds" are anticancer agents that induce apoptosis in cells.

[0175] As used herein, the term "purified" or "to purify" refers to the removal of undesired components from a sample. As used herein, the term "substantially purified" refers to molecules (e.g., polynucleotides, polypeptides, chemical compounds (e.g., gossypol compounds)) that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated. For example, an "isolated polynucleotide" is therefore a substantially purified polynucleotide.

[0176] As used herein, the term "genome" refers to the genetic material (e.g., chromosomes) of an organism or a host cell.

[0177] The term "nucleotide sequence of interest" refers to any nucleotide sequence (e.g., RNA or DNA), the manipulation of which may be deemed desirable for any reason (e.g., treat disease, confer improved qualities, etc.), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences, or portions thereof, of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.).

[0178] "Nucleic acid sequence" and "nucleotide sequence" as used herein refer to an oligonucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand. As used herein, the terms "nucleic acid molecule encoding,""DNA sequence encoding,""DNA encoding,""RNA sequence encoding," and "RNA encoding" refer to the order or sequence of deoxyribonucleotides or ribonucleotides along a strand of deoxyribonucleic acid or ribonucleic acid. The order of these deoxyribonucleotides or ribonucleotides determines the order of amino acids along the polypeptide (protein) chain translated from the mRNA. The DNA or RNA sequence thus codes for the amino acid sequence.

[0179] The term "gene" refers to a nucleic acid (e.g., DNA or RNA) sequence that comprises coding sequences necessary for the production of a polypeptide or precursor (e.g., proinsulin). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (e.g., enzymatic activity, ligand binding, signal transduction, etc.) of the full-length or fragment are retained. The term also encompasses the coding region of a structural gene and includes sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb or more on either end such that the gene corresponds to the length of the full-length mRNA. The sequences that are located 5' of the coding region and which are present on the mRNA are referred to as 5' untranslated sequences. The sequences that are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' untranslated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene which are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA finctions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

[0180] As used herein, the term "exogenous gene" refers to a gene that is not naturally present in a host organism or cell, or is artificially introduced into a host organism or cell.

[0181] As used herein, the term "vector" refers to any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

[0182] As used herein, the term "gene expression" refers to the process of converting genetic information encoded in a gene into RNA (e.g., mRNA, rRNA, tRNA, or snRNA) through "transcription" of the gene (i.e., via the enzymatic action of an RNA polymerase), and for protein encoding genes, into protein through "translation" of mRNA. Gene expression can be regulated at many stages in the process. "Up-regulation" or "activation" refers to regulation that increases the production of gene expression products (i.e., RNA or protein), while "down-regulation" or "repression" refers to regulation that decreases production. Molecules (e.g., transcription factors) that are involved in up-regulation or down-regulation are often called "activators" and "repressors," respectively.

[0183] The terms "homology" and "percent identity" when used in relation to nucleic acids refers to a degree of